4. Mammalian embryology and organogenesis

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Abstract: 4.1 Historical introduction : The origin of the human being is one of the major philosophical questions. One way of answering it is through an ontogenetic and evolutionary approach. Theories about the developmental origin in the mother's womb were first documented by Greek scholars in the 5th century BC (Breidbach 2015). Being dependent on the success of reproduction, fertility has triggered human thinking for a much longer period of human evolution as is documented in the art of the Paleolithic, the Mesolithic, the Neolithic, and later phases of human cultural evolution (Fig. 4.1). Fertility was manifested by the representation of stout mother goddesses with a demonstrative focus on their hips and breasts (Fig. 4.1 C) and by the presentation of erect penises in men (Fig. 4.1 G). In some cases, the process of birth was depicted as a key event of life (Fig. 4.1 D). Birth, pregnancy, and lactation of other vertebrate species were also of interest to ancient cultures and were illustrated, particularly, among species that played an important role in everyday life, such as prey and domesticated, mostly mammalian, animals (Fig. 4.1 A and I). Fertility rites, including hierogamy (Fig. 4.1 H) and sodomy (Fig. 4.1 J), are thought to have played a major role in prehistoric cults and were meant to increase the number of offspring and to guarantee their health (Frazer 1922, Campbell 1960). These attempts to understand and to influence our own development culminated in the artificial representation of homunculi in the Renaissance age, in which a human was "created" by alchemists in a jar. The mental construct of a test-tube baby (Fig. 4.1 K) was a symbol for the development of the human mind itself, which alchemists tried to raise to a higher level of cognition (Gebelein 1996, Wiesing 2004). The historical representation of homunculi also corresponds to the ideas of preformationists in the 17th century that the human being is fully present as a miniature in the male's sperm and only has to unfold to progress through embryological development to a fully formed human (Fig. 4.1 F; Hartsoeker 1694). With the rise of evolutionary thinking (Bell et al. 2010), a broad scientific discourse about the unique anatomy and development of mammalian species emerged. Mammalian embryology received particular attention in comparative anatomy and physiology because of its relevance in understanding our own biological history (Haeckel 1874,1877, Keibel 1906). Recent scientific approaches try to manipulate the early embryology of mammals, including man, to prevent diseases (Gilbert 2006) or to select for particular forms through domestication (Clutton-Brock 1999). Experimental biological studies mainly focus on model organisms that are easy to breed, have a large number of offspring, and have short reproductive cycles. For placental embryology, the house mouse Mus musculus is most often used (Cook 1965, Green 1968, Theiler 1989, Hedrich 2012). Among marsupials, the opossum Monodelphis domestica and the wallaby Macropus eugenii are two of the most important model organisms (e.g., Smith 2002, Hickford et al. 2009, Keyte and Smith 2009, Rousmaniere et al. 2010). However, recent studies have highlighted the value of nonmodel organisms for testing the taxonomic applicability of experimental studies, and more evolutionary questions are being asked that require extensive knowledge of non-model organisms all over the tree of life (Butler and Juurlink 1987, Jenner and Wills 2007, Milinkovitch and Tzika 2007, Tzika and Milinkovitch 2008), including mammals (Werneburg et al. 2013). The aim of the present chapter is to provide a general overview of the main developmental differences among the three major mammalian groups, to characterize mammals among amniotes, and to present some crucial aspects of the anatomy and physiology of mammalian embryogenesis. We cover different aspects of embryology in a chronological order. We first discuss particular attributes of monotremes, followed by marsupials and lastly placental mammals. We also focus on the developmental peculiaritiesin humans. Finally, we summarize some discussions on the potential

relationships between life history parameters and evolutionary patterns. The selection of topics is mainly anatomically oriented and reflects our personal morphological interest. It does not aim to represent an in-depth review of all aspects of mammalian embryology, nor does it aim to be complete. As new data, we present a reconstruction of the ancestral developmental sequence of external organ characters for Placentalia, which is based on a phylogenetic comparison of about 80 tetrapod species.

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4 Mammalian embryology and organogenesis

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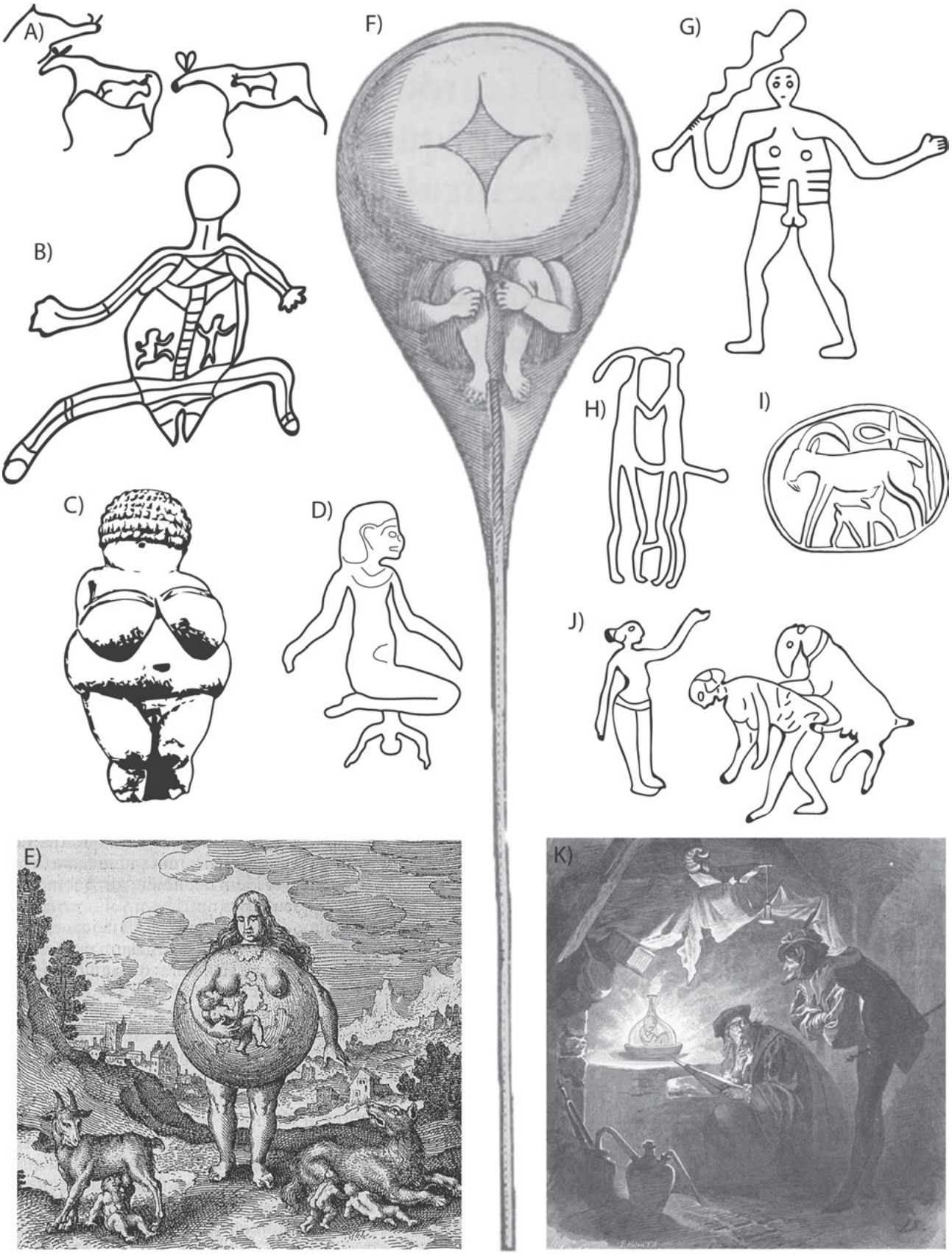
A)
B)
C)
D)
E)
F)
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I)
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K)

4.2 An overview of mammalian embryology

Mammals are distinguished from sauropsids by a number of autapomorphic characters, which are documented in stepwise evolutionary transformations in the synapsid stem lineage leading to extant mammals (Luo 2007, Chinsamy-Turan 2011). Many of these distinguishing characters relate to life history and mode of reproduction (Sánchez-Villagra 2010).

Extant mammals are characterized by the presence of complex glands in the skin including milk glands to nourish the young. Early suckling behavior of therian mammals resulted in a complex developmental modification of the skull, mainly in the areas surrounding the mouth cavity (Maier 1999). Lips and cheeks (exclusively mammalian characters) are fully formed at birth. In addition, the hyoid is also fully developed and is formed by the hyoid bone and the first branchial (first posthyoidal) arch. This structure functions as a strong cartilaginous bar at which the muscles of the tongue (hyoglossus), as well as other important muscles of the mouth and throat (e.g., sternohyoideus, thyrohyoideus, and geniohyoideus), insert. The tongue is the most important structure for suckling and is well developed in all mammalian neonates (newborns). The discovery of mammalian-like hyoids in synapsid ancestors (Kemp 2006) indicates that its general morphology is ancestral to all mammals. Furthermore, the complex mammalian larynx has important respiratory functions in altricial neonates, the development of which is enabled by the presence of three posthyoideal branchial arches, compared with only two in sauropsids.

The formation of a secondary palate can also be observed in Permian synapsids (Maier *et al.* 1996), and it most likely evolved to enable continued breathing during suckling in neonates. Although most of the secondary palate is formed by bone (hard palate), the posterior part is formed by soft tissue (velum or soft palate), which can be moved by muscles during suckling. In extant mammals, the importance of the secondary palate is highlighted by the problems that occur in neonates with misformed secondary palates.

Together, cheeks, tongue, and secondary palate restricted the movement of food inside the mouth, which may have favored the development of advanced chewing in mammals. That resulted in highly specialized postcanine teeth such as the tribosphenic molar. This specialized dentition, which is now believed to have played a very important role in the evolutionary success of mammals, is therefore probably also indirectly the result of adaptations to suckling.

Another important morphological feature of nearly all mammals is their non-kinetic skull, which allows for relatively stronger bite forces. This akinetism (such as solid fusion of the upper jaw articulation to the braincase) is related to the formation of the alisphenoid bone, which braces the surrounding elements of the skull and forms a secondary lateral skull wall (Maier 1987).

Finally, mammalian crania are also distinguished from those of other amniotes by the presence of an extensive network of facial muscles, which are innervated by the facial nerve (n. VII). In sauropsids, facialis-innervated muscles are restricted to the throat and neck area only (Maier 1999).

It has been hypothesized that specialized maternal care and an increased activity of the animal were linked to an increased anaerobic threshold and an increased aerobic activity in mammalian ancestors. Endothermy was a key feature to enable maximal aerobic metabolism. As a result, the basal metabolism of these animals would have increased, which was an essential prerequisite for the evolution of almost all derived features known for mammals. Correlated to endothermy, important transformations developed in all organ systems, which are crucial for optimized breathing (e.g., diaphragm), exploration of the environment (e.g., ear, nose, and brain anatomy), and food uptake (e.g., skull stiffening, jaw, and teeth anatomy) (Kemp 2006, Ruf 2014).

Many studies have illustrated the different anatomical transformations through ontogeny that have led to the peculiar morphotype of mammals. One of the classic examples is the evolution and development of the middle ear, which is highly derived in mammals. One auditory ossicle is derived from the ancestral tetrapod ear bone, the

◂ **Fig. 4.1: Prehistorical and historical illustrations.** (A) Two pregnant elks; rock carvings from Alta/Norway (Mesolithic age). (B) Aboriginal X-ray style figure of the pregnant Mother of Creation, Northern Territory, Australia (redrawn from a photograph). (C) Venus from Willendorf/Austria (Upper Palaeolithic, about 29,500 years old). (D) Egyptian hieroglyph of a woman giving birth; relief from the temple of Kom Ombo (redrawn from a photograph). (E) "Nutrix ejus terra est" (The Earth is his nurse) from Maier (1617/1618). (F) Homunculus in a sperm by Hartsoeker (1694). (G) Cerne Abbas Giant with erect penis, a turf-cut outline filled with chalk; Dorset/United Kindom, uncertain age. (H) Hierogamy in the rock carvings of Bohuslän/Sweden. (I) Suckling caprid; seal from Sidon/Lebanon (8th century BC), redrawn after Keel (2013). (J) Zoophilia in a rock carving at Kedareshwara Temple, Belligavi/India, redrawn from a photograph. (K) Homunculus develops in a jar; a scene from Goethe's Faust with Wagner (sitting) and Mephistopheles (von Goethe 1899).

columella (= stapes in mammals), and several other middle ear components are derived from bones of the ancestral lower jaw of early synapsids. Associated with that, a secondary jaw joint has formed between the remaining dermatocranial bones in the posterior jaw region, the dentary and squamosal (Luo 2007, Ji *et al*. 2009, Martin and Ruf 2009, Maier and Ruf 2016). Another famous case study concentrates on the ontogenetic emergence of the secondary lateral skull wall formed by the alisphenoid, which resulted from the modification of the ancestral tetrapod epipterygoid (Maier 1987).

More recent ontogenetic studies concentrate on taxonomic differences in the developmental timing of embryonic characters, and ancestral sequences of character appearance are reconstructed (Sánchez-Villagra *et al*. 2008, Weisbecker *et al*. 2008, Hautier *et al*. 2010, 2011, Werneburg *et al*. 2016, Spiekman and Werneburg 2017). One of those studies discovered an early appearance of the supraoccipital bone in the ontogeny of all mammals, which can be correlated to the increased growth and expansion of the mammalian brain when compared with non-mammalian vertebrates (Koyabu *et al*. 2014).

All those studies concentrate on the correlation between ontogenetic and phylogenetic transformations. As such, comparative studies on organismic aspects of embryology help in understanding morphological aspects of mammalian evolution.

Although embryonic development usually is defined to start with fertilization, its finalization is rather difficult to define. The term "embryology" is often restricted to intra-egg or intrauterine development of an animal, whereas birth or hatching are usually defined as the end point of embryonic life. A further distinction between embryonic and fetal developmental phases can be made prior to birth or hatching.

The start of fetal development is usually assigned to the point of development when most organs are already established and recognizable and extensive allometric growth of the animal occurs. Given the historical lack of a clear definition, we propose the disappearance of the mesencephalic vaulting of the brain in the embryo as a homologous landmark and define it as the end of embryonic development and as the start point of fetal growth (see also Werneburg *et al*. 2016). At this point in development, the brain and the bones of the cranial vault are well developed; i.e., the frontal and parietal bones of both body sides have started closure.

However, both during fetal development and after hatching or birth, organs such as claws and hairs in mammals continue to emerge, making the end point of development hard to define.

At birth or hatching, most mammals are extremely immature (Tabs. 4.1–4.3). Compared with other vertebrates, one can state that, in most cases, development continues after birth through the processes of lactation and weaning, which can take many months. After that, the animal might still rely on the help of the mother to survive, and important ontogenetic changes still occur, including the replacement of milk teeth. The maturation of gametes can be largely delayed, and some other permanent organs first develop at or after sexual maturation (e.g., antlers). To a certain extent, the animals still grow after reaching sexual maturation. In contrast to sauropsids, however, growth usually stops at a particular age in mammals, a fact that can be recognized via the fossil record (O'Meara and Asher 2016).

We follow a holistic organism concept, in which ontogeny is a process that starts at fertilization and ends with the death of the organism (Maier 1999, Maier and Werneburg 2014). An organism has to be understood as an integration of different organs and processes that change their characteristics over time. Factors that influence those changes include anatomical, functional, and ecological demands, which need to be considered in any integrative morphological and evolutionary study. As such, in the present overview on mammalian "embryology", we consider different aspects that influence embryonic development such as reproduction-related morphology of the parents and early life of the organisms after hatching or birth. Body size, litter size, and gestation length are dependent on different anatomical and physiological conditions of the mother, which also need to be considered for a comprehensive discussion on mammalian embryology.

Although embryonic or fetal development is usually concealed to general human awareness, postnatal life is more obvious. Three major extant mammalian groups can be distinguished. The Monotremata (i.e., as crown group of "Prototheria") form the sister group to Theria, which are composed of Marsupialia (i.e., as crown group of Metatheria) and Placentalia (i.e., as crown group of Eutheria). The three groups drastically differ in their mode of reproduction and life history (Tabs. 4.1–4.3). The first and most obvious difference is that monotremes have an oviparous (egg-laying) lifestyle, whereas marsupials and placentals are viviparous (live bearing). Second, marsupials have extremely immature so-called altricial neonates, whereas the other groups show a slightly (monotremes) or largely (placentals) more developed anatomy at birth with precocial groups as extremes among Placentalia. The terms altriciality (e.g., naked and blind and dependent on parents for food) and precociality (e.g., early independent, nidifugous) were coined by Haeckel (1866) and are

Tab. 4.1: Comparison of important life history features in sauropsids and the three major mammalian groups.

Taxon	Sauropsida (in general)	Monotremata	Marsupialia	Placentalia
Extant diversity	>20.000 described species	1 platypus, 4 echidna species	>330 described species	>4.500 described species
Endothermic	Only Aves (possibly some non-avian dinosaurs)	Yes	Yes	Yes
Cloaca	Yes	Yes	No	Rare
Perineum	No	No	Yes	Yes
Reproduction	Primarily oviparous (egg laying)	Oviparous (egg laying)	Viviparous (live birth)	Viviparous (live birth)
Scrotum	No	No	Yes	Yes
Eggs	Polylecithal	Polylecithal	Alecithal	Alecithal
Cleavage	Meroblastic-discoidal	Meroblastic-discoidal	Holoblastic-rotational	Holoblastic-rotational
Placenta	Some squamates	No	Yes	Yes
Placenta type	If existing: choriovitelline	–	Mainly choriovitelline	Choriovitelline and/or allantochorial
Embryonic nutrition	Yolk, placenta in some squamates	Histotrophs, yolk	Histotrophs, placenta	Placenta
Gestation length	Medium	Short	Short	Long
Maturity level at delivery	Generally precocial	Altricial	Altricial, neonates have to crawl to mother's teat	Wide range from altricial to precocial neonates
Mammary glands	No	Yes	Yes	Yes
Teats	No	No	Yes	Yes
Uniform milk composition during lactation	–	Yes	No	Yes
Pouch	No	Only in echidna	Yes	No
Pouch bones	No	Yes	Yes	No
Continued growth throughout life	Yes	No	No	No

derived from the Latin words altrix (i.e., foster mother or dependent on care) and praecox (i.e., precipitate, in relation to the mechanisms of the nervous system and the locomotory apparatus).

After birth or hatching, mammals show a great investment in parental care. This is particularly visible in the form of guarding and lactation. After weaning, food is often supplied to the young.

Early development of monotremes is difficult to study because the animals only have a small litter size of one or two young. Nowadays, they are protected by national conservation laws in Australia and New Guinea, and it is difficult to access new embryos. One famous researcher of monotreme development was Richard Semon (1859–1918) from Jena, Germany (Hoßfeld and Olsson 2003a), who collected a large egg sample of the short-beaked echidna *Tachyglossus aculeatus* and described the early development of this species (Semon 1894a–d, 1897a, b, 1904; expanded by Werneburg and Sánchez-Villagra 2011). This material was used as a source for further developmental studies of particular organs (e.g., Klaatsch 1895). Detailed studies on the earliest development were performed by James Peter Hill (1873–1954), whose monotreme and marsupial collections are currently stored at Museum für Naturkunde Berlin, Germany (Richardson and Narraway 1999, Giere and Zeller 2006).

In their reproductive biology, monotremes represent a mosaic of ancestral amniote, derived mammalian, and unique monotreme characters (Griffiths 1989, Werneburg and Sánchez-Villagra 2011), as mirrored in discoveries of whole-genome studies (Warren *et al.* 2008). Like all amniotes, therian mammals have an internal fertilization, but they evolved a specialized reproductive system. Therian cleavage is unique because it develops a specialized cell population in the embryo that allows integration in the uterine tissue of the mother. With this integration, a new organ, the placenta, is formed that permits elongated embryonic development in the mother's womb. Intrauterine development enables the viviparity of therian mammals. No extrauterine egg is needed.

Like placental mammals, marsupials are viviparous. However, they give birth to highly altricial and immature young and much of organogenesis occurs during lactation. The gestation, during which maternal nutrition

is supplied by a placenta, is very short (1–2 weeks). To overcome the extreme conditions to which the neonate is exposed at birth, marsupials evolved highly specialized anatomical adaptations.

In contrast to marsupials, placental mammals have, on average, a relatively longer gestation length, and the young are born at a more developed stage. There is a wide range between altricial and precocial conditions at birth. However, all placentals are born at a more developed stage than marsupials, so there is no overlap between the altricial state found in marsupials and that in placentals. Placentals are characterized by a great extant diversity and include arboreal, fossorial, terrestrial, gliding, flying, diving, and permanently aquatic forms. All morphological adaptations are mirrored by the relative timing of related prenatal character development (Müller 1973). These changes in timing result in different relative sizes and degrees of differentiation in the adults (Werneburg *et al.* 2015). For example, the fossorial mole or the bats develop their specialized and large forelimbs relatively early when compared with other placental mammals (Sears 2006, 2008, Richardson *et al.* 2009, Mitgutsch *et al.* 2012, Bickelmann *et al.* 2012, Koyabu *et al.* 2014).

Relatively few organic changes appear in later phases of life (e.g., aging processes and temporary changes) (Portmann 1944), although changes in bone density and shape can be traced quantitatively (e.g., Wilson 2011, Urban *et al.* 2016). These aspects of postnatal development are not discussed in the present overview.

4.3 Reproduction and related organs

Unlike marsupials and placentals, monotremes are oviparous. Like sauropsids (and a few placental mammals), they have a cloaca (Romer 1976). The cloaca is a single opening ("Monotremata" from Greek μόνος and τρῆμα, "single hole") in which feces, urine, and eggs are deposited before leaving the body. During embryonic development of therian mammals, the opening of the urogenital system gets separated from the anus (Fig. 4.2 A–D) (Liem *et al.* 2001). Monotremes show an intermediate anatomy in this regard. Although they possess a cloaca (Fig. 4.3 D), like sauropsids, the proximal part is separated into a rectal region (coprodaeum) and a more ventral urodaeum, which contains the urinal and genital products (Fig. 4.2 E–F and Q). Therian mammals have a perineum, which separates the anus and the urogenital tract (Fig. 4.2 G, L, and R–S).

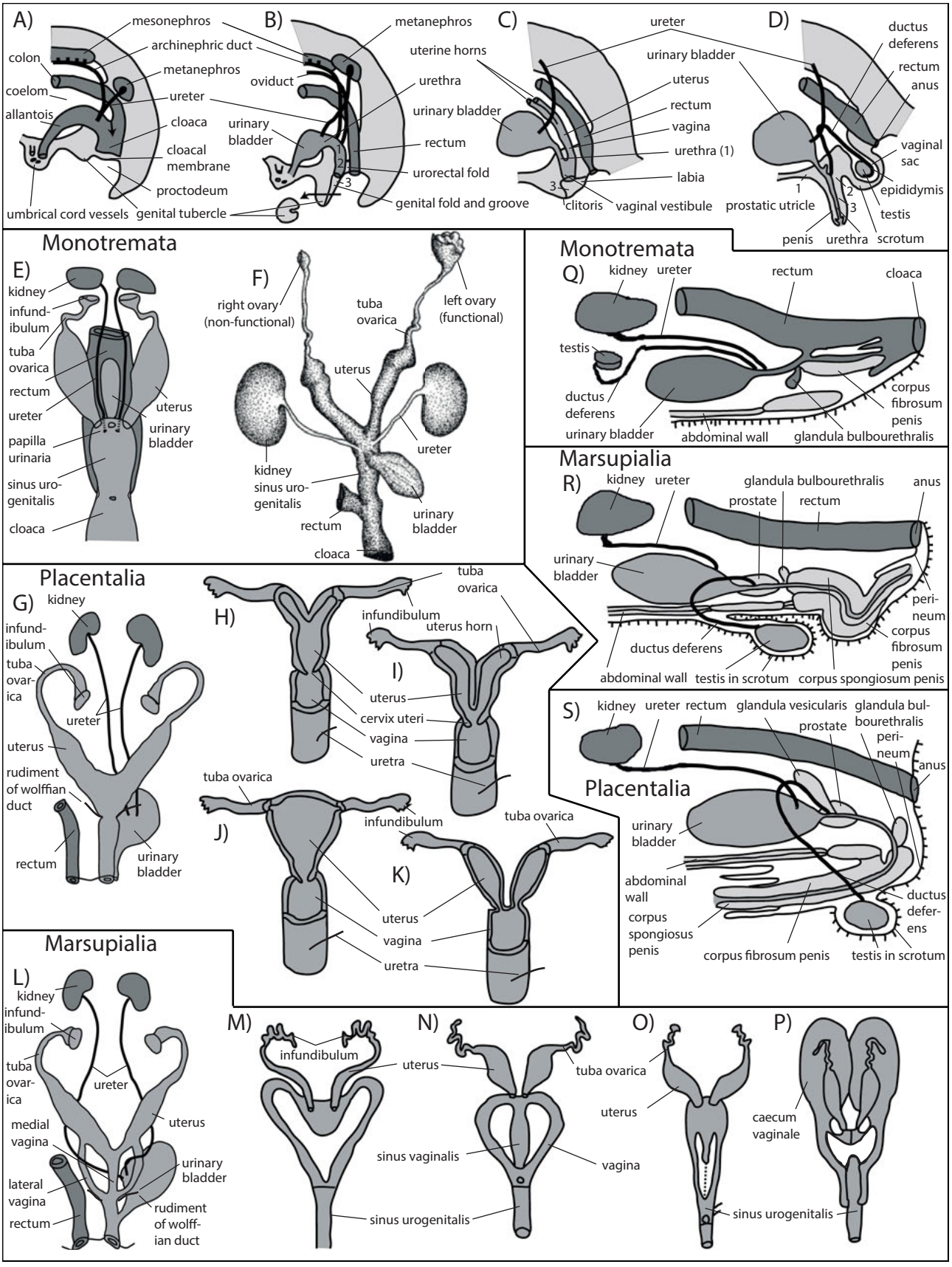
In marsupials, a small diverticulum represents the last trace of the cloaca. It is fully reduced in most placental mammals (Riedelsheimer *et al.* 2007), and the coprodaeum (cranial-most part of the ancestral cloaca) becomes the terminal part of the gut and opens into the anus (Fig. 4.2 S).

In monotremes and marsupials, the whole urogenital system is paired. The ovaries of the female monotremes resemble those of the sauropsids. In the monotreme species *Ornithorhynchus anatinus*, the platypus, only the left ovary is functional (Fig. 4.2 F; in birds the right one is functional). In the right oviduct, which is about the same size as the left one, the gamete only reaches the ovocyst stage (Zeller 2004a).

In the monotreme species *T. aculeatus*, the short-beaked echidna, both ovaries are evenly developed (Fig. 4.2 E). The egg is transported to one of the paired uteri, which open separately into the sinus urogenitalis that enters the cloaca (Greven 2004). The egg then enters the single urogenital sinus and then the cloaca (Fig. 4.2 E). A vagina is absent.

In marsupials, the paired uteri open into the vaginal sinus (Fig. 4.2 L–P). The Müllerian ducts do not fuse during development, which results in two vaginas (didelphy) (Starck 1995). Their posterior parts are fused in certain groups (Macropodidae). The anterior parts of the vaginae are sometimes fused to an unpaired sinus vaginalis and enter a united urogenital canal (Fig. 4.2 N; Greven 2004). In peramelids, the sinus vaginalis forms an extended anterior diverticulum ("caecum vaginalia"), which can serve as a spermatozoa reservoir (Fig. 4.2 P). In many forms,

▸ **Fig. 4.2: Reproductive organs.** (A–D) Lateral diagrams of the division of the embryonic cloaca in placental mammals (sexually homologous urethral segments are identified by the same number): (A–B) early and later sexually indifferent stages, (C) differentiation of the female, (D) differentiation of the male. (E) Genital apparatus of the female short-beaked echidna, *Tachyglossus aculeatus*. (F) Female organs of the monotreme platypus, *Ornithorhynchus anatinus*. (G–K) Genital apparatus of female placental mammals: (G) general structure of the female apparatus in a placental mammal, (H) uterus bicornis, (I) uterus bipartitus, (J) uterus simplex, (K) uterus duplex. (L–P) Genital apparatus of female marsupial mammals: (L) general structure of the female apparatus in a marsupial mammal; vaginal modification in (M) the large American opossum *Didelphis*, (N) the kangaroo *Macropus*, (O) the rat-kangaroo *Hypsiprymnodon*, and (P) bandicoots (Peramelidae). (Q–S) Genital apparatus of males: (Q) monotreme, (R) marsupial, (S) placental mammal condition. A–D after Liem *et al.* (2001); E, G, L after Starck (1995) and Mickoleit (2004); F after Grant (2013); H–K after Romer (1976); M–P after Starck (1995), Pough *et al.* (2012), and Mickoleit (2004); Q–S after Mickoleit (2004) and Liem *et al.* (2001).


A)
mesonephros
archinephric duct
colon
metanephros
coelom
ureter
allantois
cloaca
cloacal membrane
proctodeum
umbrical cord vessels
genital tubercle
B)
metanephros
uterine horns
oviduct
urethra
urinary bladder
rectum
urorectal fold
genital fold and groove
C)
ureter
urinary bladder
uterus
rectum
vagina
urethra (1)
labia
clitoris
vaginal vestibule
D)
ductus deferens
rectum
anus
vaginal sac
epididymis
testis
prostatic utricle
penis
urethra
scrotum
Monotremata
E)
kidney
infundibulum
tuba ovarica
rectum
ureter
uterus
urinary bladder
papilla urinaria
sinus urogenitalis
cloaca
F)
right ovary (non-functional)
tuba ovarica
left ovary (functional)
uterus
ureter
kidney
sinus urogenitalis
urinary bladder
rectum
cloaca
Monotremata
Q)
kidney
ureter
rectum
cloaca
testis
ductus deferens
urinary bladder
abdominal wall
corpus fibrosum penis
glandula bulbourethralis
Marsupialia
R)
kidney
ureter
glandula bulbourethralis
anus
prostate
rectum
urinary bladder
perineum
ductus deferens
corpus fibrosum penis
abdominal wall
testis in scrotum
corpus spongiosum penis
Placentalia
G)
kidney
infundibulum
tuba ovarica
ureter
uterus
rudiment of wolffian duct
rectum
urinary bladder
H)
tuba ovarica
infundibulum
uterus horn
I)
uterus
cervix uteri
vagina
uretra
tuba ovarica
J)
infundibulum
tuba ovarica
K)
uterus
vagina
uretra
S)
kidney
ureter
rectum
glandula vesicularis
glandula bulbourethralis
prostate
perineum
anus
Placentalia
urinary bladder
abdominal wall
corpus spongiosus penis
ductus deferens
corpus fibrosum penis
testis in scrotum
Marsupialia
L)
kidney
infundibulum
tuba ovarica
ureter
medial vagina
uterus
urinary bladder
lateral vagina
rectum
rudiment of wolffian duct
M)
infundibulum
N)
uterus
tuba ovarica
sinus vaginalis
vagina
sinus urogenitalis
O)
uterus
P)
caecum vaginale
sinus urogenitalis

the sinus vaginalis continues caudally until it reaches the urogenital duct. It breaks through that duct before birth and forms a permanent or, in most cases, a temporary birth canal (third or pseudovagina). Most marsupials have a seasonal reproduction period and have more than one period of estrus per year (polyoestrous).

Within placental mammals, the vagina is uniform and there is a trend to unite both uteri (Fig. 4.2 G–K). As such, the uteri can open separately (Fig. 4.2 K; uterus duplex: Rodentia, some Chiroptera) or with only one opening (Fig. 4.2 G–J) into the vagina. Depending on the degree of fusion, some placental mammals have a uterus bipartitus (Fig. 4.2 I; most carnivorans, some chiropterans), a uterus bicornis (Fig. 4.2 H; Eulipotyphla, some chiropterans, Cetartiodactyla), or – after total fusion – a uterus simplex (Fig. 4.2 J; some chiropterans, some xenarthrans, most primates). The sinus urogenitalis is largely reduced in placentals (Greven 2004). In primates and some rodents, urinary and genital tracts open separately. The vagina is unpaired (monodelphy) and opens into a vulval vestibule (vestibulum vaginae) (Storch and Schröpfer 2004).

Catarrhine primates, including humans, have a menstruation cycle, which involves regular bleedings in a rhythm of about 1 month (species specific). Bleeding derives from the uterine mucosa, which develops at the end of each cycle if the female does not become pregnant (Starck 1995).

The reproductive tracts of monotreme males differ from most therian mammals in having no scrotum; their testes are embedded inside the abdominal cavity near the kidneys (testicondy). The penis is situated within the urogenital sinus when not erected (Fig. 4.2 Q).

Although some placental groups secondarily show testicondy, most therians exhibit a permanent or seasonal displacement of the testes outside the abdominal cavity through the inguinal canal to the outside of the body (descensus testis) (Fig. 4.2 R–S) (Storch and Schröpfer 2004, Kleisner *et al.* 2010). The displacement of the testes is most likely caused by the high body temperature, which would have a mutagenic effect on spermatogenesis (Greven 2004). Most placental mammals have their testes outside the abdomen; only Xenarthra, Cetacea, as well as Sirenia, Proboscidea, and most other Afrotheria exhibit testicondy, which presents a strong phylogenetic signal (Werdelin and Nilsonne 1999).

The testes in marsupials experience a full and permanent "descensus" and lie subcutaneously. They are mostly situated in a scrotum, which can be pediculate. Compared with placentals, scrotum and testes are situated anterior to the penis in marsupials (except for the marsupial mole *Notoryctes typhlops*). The penis lies inside a penis pocket and has a kinked shape in its dormant phase (Fig. 4.2 R). A musculus levator penis can compensate the kink and a retractor muscle originating from the sacrum can retract the muscle inside the pocket. In most marsupials, in correspondence to the paired vagina, the terminal end of the penis is bifurcated.

Like the altricial platypus (Fig. 4.12 A) and altricial birds, placental mammals partly build very complex breeding burrows and nest constructions. Among others, subterranean nests are known for moles, common hamsters, and marmots. Epigaeic nests are known for field voles and boars. Nests in the soil can be found in harvest mice and hazel dormouse. Edible dormice build nests in tree holes and squirrels have nests in tree crowns. Nests can be complex (squirrel) or simple (house mouse). The litter nests can be more comprehensive and denser than nests for sleeping (Storch and Schröpfer 2004).

4.4 Gametes

During the proestrus, in all mammals, follicle cells are formed in the ovary of the female (Fig. 4.4 B), which produce estrogens and induce the estrus (heat). Subsequently, ovulation takes place. The empty follicle is folded and forms the corpus luteum (yellow body). It produces progesterone and is necessary to maintain pregnancy in females. After ovulation, the follicle is folded, and it forms a true corpus luteum (yellow body of the ovary), which is active throughout the whole intrauterine phase of development. Size increase is only facilitated through cell growth and not through cell division.

As in sauropsids, the eggs of monotremes are polylecithal, sometimes referred to as macrolecital, meaning that a large amount of yolk is present in the egg (Greven 2004). Inside the oviduct, tertiary egg layers are developed around the fertilized egg (zygote). These are homologous to those of the sauropsid egg (Fig. 4.5 G).

The primary membrane of the ovum is the vitelline layer, which is called zona pellucida in mammals (Fig. 4.4 Db-1). One of the glycoproteins of this layer is an important binding site for the spermatozoa during the acrosomal reaction.

The secondary layer, the theca folliculi, is formed by follicle cells (Fig. 4.4 B) in one or two sublayers. The theca externa consists of large and polygonal cells, which are separated from the theca interna by a basal membrane; both are formed by the stroma ovarii. The lutein cells are solely formed by follicle cells. Like in sauropsids, the follicle does not form a cavity in monotremes (Starck 1995).

The tertiary layer, the actual eggshell and albumen, is formed by secretions of the oviduct and uterine glands

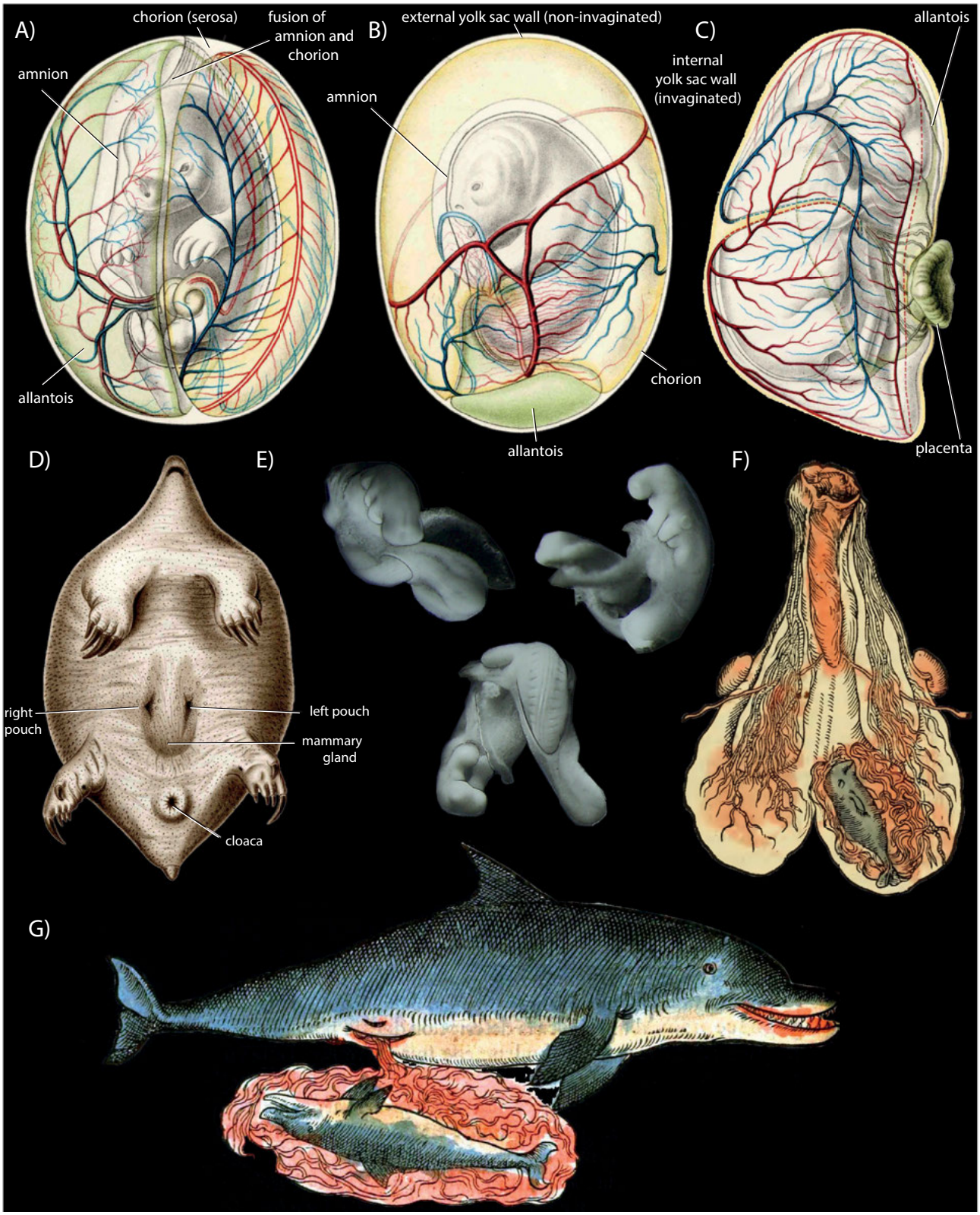


Fig. 4.3: Historical images on mammalian development. (A) Embryo of the monotreme short-beaked echidna *Tachyglossus aculeatus* within its extraembryonic membranes. (B) Embryo of the koala *Phascolarctos cinereus*, slightly shifted position within the yolk sac. (C) Late rabbit embryo inside its extraembryonic membranes; chorion not shown. (D) External sexual organs of a female *T. aculeatus*. (E) Embryo of *T. aculeatus* with a coiling trunk in three different views. Common bottlenose dolphin *Tursiops truncatus* in utero (F) and at birth (G). A–C, after Semon (1894b), D after Klaatsch (1895), E, from Embryological collection Berlin (M153); see also Werneburg and Sánchez-Villagra (2011). F and G, after Belon (1551).

(Zeller 2004b). The external layers form the shell and are parchmentlike in monotremes (containing keratin).

At a total diameter of 15 mm and an ovum size of 5 mm, monotreme eggs are relatively smaller and have less yolk when compared with the eggs of sauropsids. However, compared with those of therian mammals, they are enormous in size. During ovulation, the egg grows to up to four times its initial size. In contrast to sauropsids, the egg is still growing during the passage through the genital tract by receiving nutrition in the form of embryotroph and uterine milk (histotrophy) (Storch and Schröpfer 2004).

In monotremes, the albumen layer of the egg is shed shortly before fertilization and then serves to trap spermatozoa. The eggshells of monotremes and marsupials are very dense and spermatozoa cannot penetrate them. Therefore, insemination has to occur before shell formation (Greven 2004). After fertilization, the egg is quickly transmitted through the oviduct and fertilization stages can be found in the uterus (Starck 1995).

In contrast to those of other amniotes, therian follicle cells form a cavity (Fig. 4.4 B-1), which appears to be related to size reduction and the transition from poly- to alecithal eggs (Starck 1995). The tenrecid placentals are an exception in this regard (Strauss 1938, Enders *et al*. 2005).

Marsupial eggs are covered by a hull of albumen and shell for over two thirds of their intrauterine development but are nourished by uterine secretions during the final stages of intrauterine development (Fig. 4.4 Da). The eggshell may serve as an immunological barrier to the uterus. The external albumin layer of the marsupial egg is homologous to that of monotremes (Zeller 2004b) and consists of mucopolysaccharide. An external, keratinized shell membrane forms in the uterus. The corpus luteum is fully developed three days after ovulation and persists during the whole lactation phase.

The eggs of therian mammals are relatively small with a diameter of about 0.1 to 0.2 mm (human: 0.15 mm).

In general, the number of egg cells during one ovulation corresponds with the maximum number of offspring per litter in mammals. However, in few species (e.g., the elephant shrew *Elephantulus*), up to 120 eggs are released but only one or two are implanted in the uterus. Moreover, the plains viscacha *Lagostomus maximus* (Rodentia) produces 300–800 eggs (Weir 1971).

Polyembryony is known in two armadillo species (*Dasypus hybridus* et *novemcinctus*), in which only one egg ovulates (with one corpus luteum), and through division of one blastocyst, four or nine embryos of the same sex develop (Fernandez 1909, Newman and Patterson 1910).

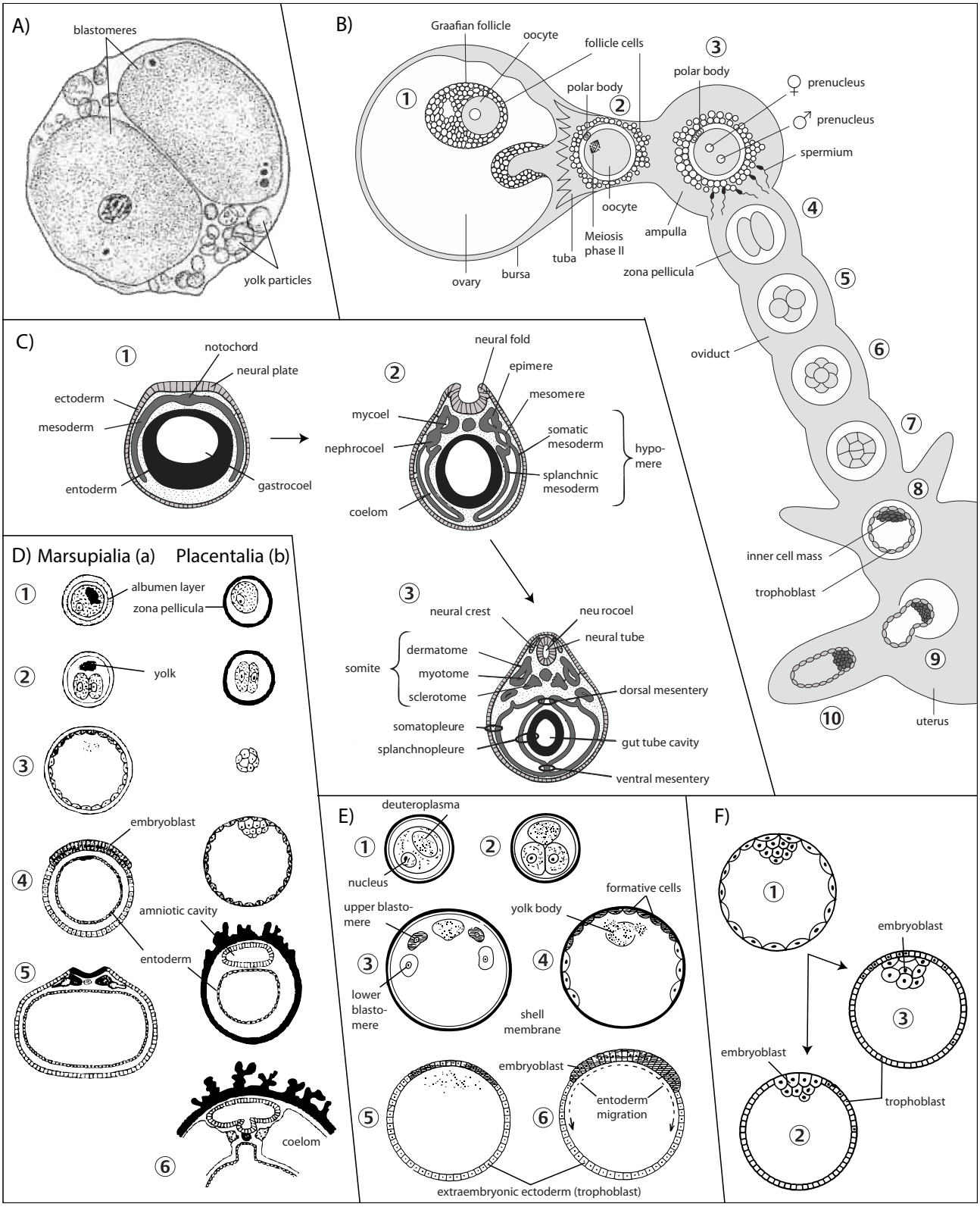
Male gametes can be produced throughout the entire lifecycle, whereas all female gametes are developed around birth in mammals. As in sauropsids, sperm of monotremes have a long, filamentous head (Storch and Schröpfer 2004). In contrast to sauropsids and some marsupials, however, no physiological polyspermy (egg fertilization with more than one sperm) is found in monotremes (Starck 1995).

The spermatozoans have disklike "heads" in marsupials (partly barlike in the quoll *Dasyurus*) and have two processes at their terminal ends, which are deeply invaginated at the insertion site of the middle part. The terminal part of the sperm "tail" is very short and thin. Mature spermatozoa of South American marsupials (Didelphidae and Caenolestidae) attach to each other and form units while swimming. They separate again in the oviduct, where fertilization takes place.

4.5 Blastogenesis

Monotremes show a meroblastic (incomplete) type of cleavage (Caldwell 1884), which is an ancestral amniotic feature. A large amount of yolk is concentrated at one

▸ **Fig. 4.4: Early development in mammals.** (A) Yolk elimination in the form of small yolk particles in the opossum *Didelphis*. (B) Early embryonic development in the house mouse *Mus musculus* (Placentalia) until implantation. Graafian follicle with oocyte (1), ovulation (2), fertilization (3), cleavage: 2-cell stage (4), 4-cell stage (5), morula (6), compaction of morula (7), blastocyst (8), hatching (9), and implantation (10). (C) General steps in successive differentiation of the mesoderm and the neural tube in vertebrates. Mesoderm initially comes to lie between ectoderm and entoderm, and neurulation begins with a dorsal thickening of the ectoderm into a neural plate (1). Mesoderm differentiates into three major regions: epimere, mesomere, and hypomere, and the neural plate folds (2). Each mesoderm layer gives rise to specific layers and populations of mesodermally derived cell populations. The neural folds fuse and form a hollow neural tube, and neural crest cells emerge from the edges of the original neural plate (3). (D) Comparison of early ontogenetic processes of Marsupialia and Placentalia: yolk elimination, blastocyst formation (1–3), yolk sac formation (4a), entypy of the embryoblast (4b), mesoderm formation (5a, 6b), trophoblast expansion, and formation of exocoele (5b). (E) Cleavage and yolk elimination in marsupial mammals (quoll, *Dasyurus*). Non-fertilized egg cell with yolk vacuoles (1); 2-cell stage, the yolk vacuole is eliminated (2); 8-cell stage, formation of trophoblast cells (3); formation of blastula, the yolk body is resorbed (4); further development of the blastocyst (5–6). (F) Differentiation of the blastocyst in therian mammals. Blastocyst (1); early integration of the embryoblast (i.e., inner cell mass) into the trophoblast in Marsupialia (2); entypy: trophoblast grows over embryoblast in Placentalia (3). A, after Starck (1975); B, after Wehner and Gehring (2007); C, after Kardong (2008); D and F, after Starck (1995); E, after Starck (1975).


A)
blastomeres
yolk particles
B)
Graafian follicle
oocyte
follicle cells
polar body
polar body
prenucleus
prenucleus
spermium
oocyte
Meiosis phase II
ampulla
tuba
ovary
bursa
zona pellicula
oviduct
inner cell mass
trophoblast
uterus
C)
notochord
neural plate
ectoderm
mesoderm
entoderm
gastrocoel
neural fold
epimere
mesomere
mycoel
nephrocoel
somatic mesoderm
splanchnic mesoderm
hypo-mere
coelom
neural crest
neu rocoel
neural tube
somite
dermatome
myotome
sclerotome
dorsal mesentery
somatopleure
splanchnopleure
gut tube cavity
ventral mesentery
D) Marsupialia (a) Placentalia (b)
albumen layer
zona pellicula
yolk
embryoblast
amniotic cavity
entoderm
coelom
E)
deuteroplasma
nucleus
upper blasto-mere
lower blasto-mere
yolk body
formative cells
shell membrane
embryoblast
entoderm migration
extraembryonic ectoderm (trophoblast)
F)
embryoblast
embryoblast
trophoblast

Tab. 4.2: Comparison of selected life history traits in monotreme and marsupial mammals. Mainly summarized after Hayssen *et al.* (1993).

Major taxon	Species	Common name	Gestation length	Litter size	Maturity level at birth	Eye opening after birth	Weaning	Neonatal mass	Neonatal size	Sexual maturity
				Monotremata						
Tachyglossidae	*Tachyglossus aculeatus*	Echidna	27 days	1 or 2	Altricial	?	~200 days	378–380 mg	12–17 mm	~1 year
Ornithorhynchidae	*Ornithorhynchus anatinus*	Platypus	12–30 days	2	Altricial	>11 weeks	3–4 months	?	~25 mm	1–2 years
				Marsupialia						
Didelphidae	*Monodelphis domestica*	Grey short-tailed opossum	14–15 days	1 to 11	Altricial	2–5 weeks	49–56 days	100 mg	10 mm	3–7 months
Didelphidae	*Didelphis virginiana*	Virginia opossum	11–13 days	6 to 14	Altricial	50–72 days	80–104 days	130–160 mg	14 mm	6–8 months
Dasyuridae	*Dasyurus viverrinus*	Eastern quoll	~20 days	1 to 6	Altricial	72–91 days	5.5–6 months	20 mg	4.7–7.6 mm	11 months
Dasyuridae	*Sminthopsis crassicaudata*	Fat-tailed dunnart	13–18 days	6 to 9 (down to 1 rare)	Altricial	48–50 days	~70 days	~10 mg	3.5–4 mm	100–160 days
Peramelidae	*Perameles nasuta*	Long-nosed bandicoot	11–13 days	1 to 6	Altricial	44–49 days	~67–75 days	237 mg	12.8 mm	~4 months
Peramelidae	*Isoodon macrourus*	Northern brown bandicoot	12 days	1 to 7	Altricial	44–49 days	59 days	188.3 mg	13.83 mm	~4 months
Tarsipedidae	*Tarsipes rostratus*	Honey possum	21–28 days	1 to 4	Altricial	56 days	90 days	4.3 mg	?	5–6 months
Macropodidae	*Macropus rufus*	Red kangaroo	31–33 days	1	Altricial	115–150 days	~1 year	817 mg	?	1.5–3 years
Phascolarctidae	*Phascolarctos cinereus*	Koala	25–36 days	1 (2 occasional)	Altricial	?	10–12 months	360 mg	?	2–3 years

pole (i.e., telolecithal, from Greek τέλος, telos, meaning "end"), and only the animal pole shows cleavage (Storch and Schröpfer 2004). This is similar to the cleavage mode of fishes, sauropsids, and cephalopods (Fioroni 1987). Cleavage already takes place inside the uterus. The early embryo shows a so-called discoidal cleavage, which results in a flat blastodisk. The blastomeres (i.e., dividing cells) are attached to the yolk and separate themselves by strangulation and not by horizontal cleavage division. From the margin of the blastodisk, some cells (vitellocytes) migrate to the yolk. Later on, they fuse and form a syncytium, which surrounds the blastodisk.

The cleavage in therian mammals is total and equatorial (i.e., rotational cleavage). Therian mammals show holoblastic development, i.e., all cells show cleavage. In this regard, it mirrors more the non-amniote vertebrate than the sauropsid development. In contrast to other vertebrates with holoblastic development (in which both yolk and embryo cells show cleavage), early therian development occurs inside the mother. Therefore, the ancestral food supply via yolk cells is reduced, because much of the nutrition comes from the placenta. The development is hence secondarily holoblastic, as only embryonic cells show cleavage. In fact, this is rudimentary meroblastic cleavage because it is derived from the ancestral amniote mode of oviparous, meroblastic reproduction (Starck 1995).

Inside the blastocyst of marsupials, yolk particles are present, which are eliminated from the egg during the first cleavage stage (Fig. 4.4 A and D–E). They persist for a while as a yolk body between the blastomeres in most species until they are disintegrated and digested (phagocytosed) by blastocyst cells (Blüm 1986, Greven 2004).

Cleavage itself is relatively consistent in marsupials. First, a meridian cleavage takes place (radial orientation of cells). The fourth cleavage step is horizontal and results in the formation of two cell rings, which are superimposed upon each other. In contrast to placental mammals, no morula is formed.

In contrast to placentals, gestation and lactation do not result in the suppression of the estrous cycle in marsupials, and it is important to note that the estrous cycle is longer than gestation (Tyndale-Biscoe 2005). Directly after giving birth, kangaroo females can conceive again. If one kangaroo young is still in the pouch, the cleavage of a new embryo stops at the 100-cell stage (diapause) (Renfree 1993, Renfree and Shaw 2002, Hickford *et al.* 2009), and it continues to develop when the older sibling leaves the pouch.

Usually, placental mammals have oligolecithal eggs with no or only very little yolk inside (Starck 1995). The albumen layer is missing in placental mammals (except for rabbits, *Oryctolagus cuniculus*).

Cleavage of placental mammals is extremely slow and is not radial (Fig. 4.4 B and Db). The first cleavage is meridional (Fig. 4.4 B-4). In the second cleavage, one of the blastomeres shows a meridional cleavage, the other one an equatorial cleavage (Fig. 4.4 B-5), and so on. The cells do not separate completely from each other (Blüm 1986). Beginning with the 16-cell stage, the blastomeres form a morula (mulberry) inside the uterus (Fig. 4.4 B-6), which has the size of a pinhead and is comparable with the blastula of monotremes. Subsequently, however, the cells undergo a compaction with the help of adhesion molecules (Fig. 4.4 B-7). During the subsequent multiplication of cells, a blastocyst develops with a fluid filled cavity (blastocoel) inside.

4.6 Gastrulation

During gastrulation, three germ layers emerge from the blastocyst: ectoderm, mesoderm, and entoderm (Figs. 4.4 C and 4.5 A, C, I). The ectoderm differentiates into organs and tissues such as the epidermis, the brain (through neurulation), and the facial skeleton (Hall 2009). The mesoderm develops into the heart, blood vessels, and most musculature, among other tissues. The entoderm develops into the gut, gut-related organs such as the liver, and other structures. All three germ layers can participate in the formation of extraembryonic membranes, which variably contribute to the placenta.

During cleavage, a layer of eight cells forms in monotremes. After closure of the syncytial ring (i.e., an outer layer of multinucleate cells), and through active cell migration, early growth of the yolk and a flattening of the blastocyst occur. The so-called blastoderm is able to ingest the maternal nutrients inside the uterus (Zeller 2004b). Now, a subgerminal cavity is present between embryo and yolk. This blastoderm of monotremes is a bit larger than in sauropsids and already envelops the yolk at the beginning of gastrulation (Blüm 1986). Around the yolk-navel, the syncytial ring now shrinks into a plasma mass. This process is finished before the primitive streak is formed.

Early in monotreme development, two cell layers are distinguishable in the blastoderm. The external one consists of large cells, the prospective ectoderm cells. The layer of smaller cells will become entoderm cells. Later on, both layers integrate in the unilaminar blastoderm.

Afterward, the entoderm cells amoeboidally migrate down and condensate into the entoderm (hypoblast); the ectoderm cells stay to form the ectoderm (epiblast) (Starck 1995).

In this bilaminar stage, the embryonic body begins to form with the appearance of a primitive streak at the posterior end of the embryo. Always separated from it, a primitive knot develops in the center of the embryo. Here the invagination of the primitive gut (archenteron) takes place. Around the primitive knot, which is homologous to the primitive plate of sauropsids, the chordal plate (dorsomedial), the protochordal plate (rostal), and the gastral mesoderm (lateral) form. Formation of gastral mesoderm occurs earlier in monotremes than in sauropsids. The thickened ectodermal area anterior to the primitive knot/plate (Keimschild), however, appears earlier in reptiles.

The primitive streak of monotremes serves as an independent area of mesoderm formation and is homologous to the primitive streak found in therian mammals (Starck 1995).

The further tissue formation of monotremes (neurulation, somite formation, and schizamnion) is similar to the processes of all amniotes (Starck 1995), but differences occur in the relative timing of organ appearance and growth rates.

In marsupials, one cell ring of the forth cleavage stage forms the embryonic part (embryoblast) and the other ring forms the extraembryonic ectoderm (trophoblast). The embryoblast (i.e., formed by blastomeres) is parietal and forms a one-layered protoderm. This protoderm is often called blastula because it is embedded in its epithelium and no primitive knot is formed. However, because a major part of its parietal wall persists as extraembryonic ectoderm, the term "blastocyst" is more adequate (Starck 1995). It takes about 1 to 2 weeks from fertilization to blastocyst formation. Afterward, the embryo continues intrauterine development for a further 1 or 2 weeks until birth (Zeller 2004a).

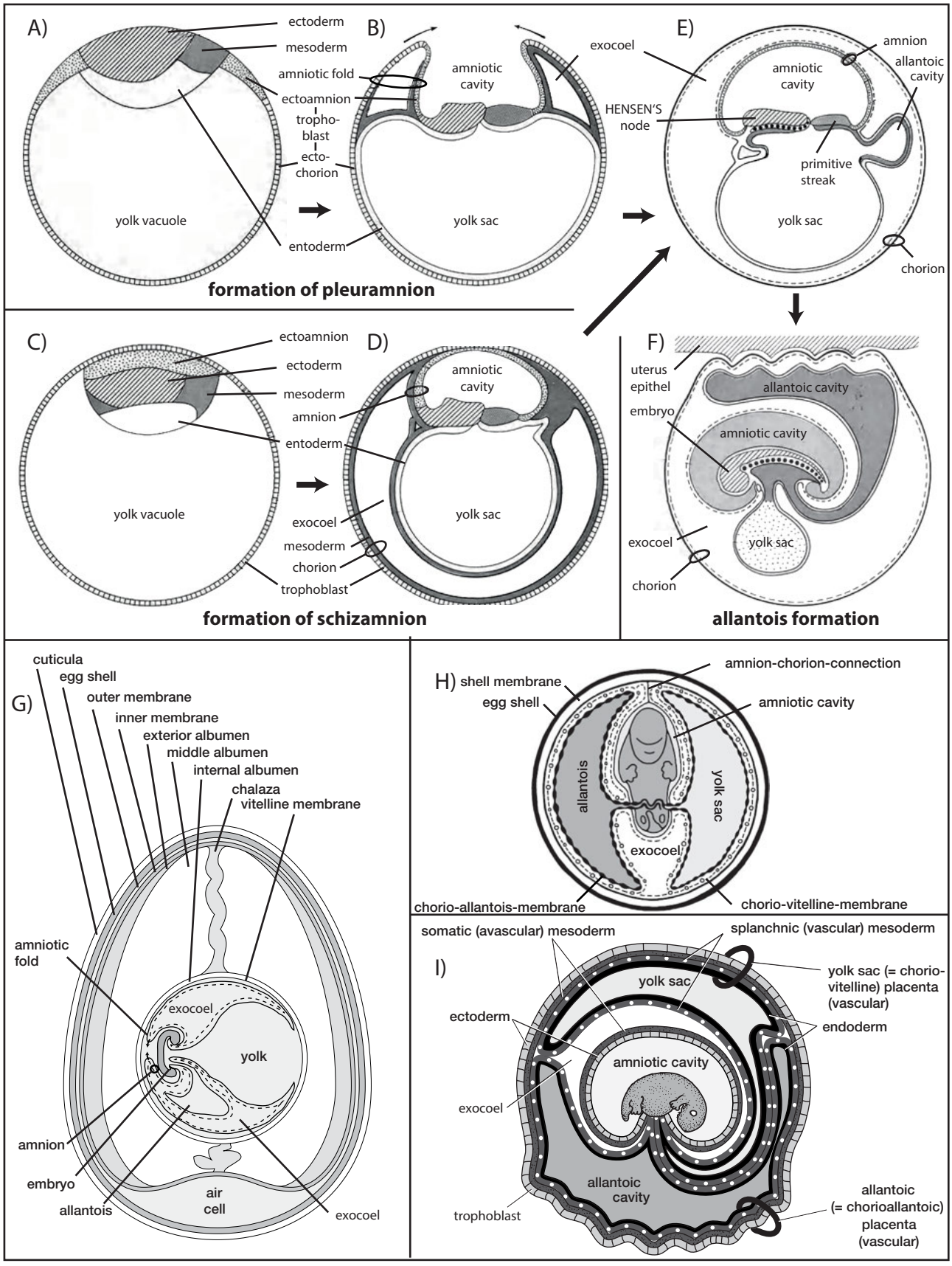
In contrast to Placentalia, no compaction (morula) of the embryoblast takes place, and the trophoblast does not grow over the embryoblast (i.e., no entypy) in marsupials. Consequently, the embryoblast is never covered by the trophoblast in marsupials (Fig. 4.4 E-4-6 and F2-3) (Starck 1995, Lillegraven 2003, Greven 2004, Zeller 2004a). The unilaminar embryoblast (blastocyst layer) separates entoderm cells. The now bilaminar blastocyst forms the neural plate with a primitive streak.

Through entypy of embryoblast cells, the morula of placental mammals differentiates into two distinct parts, the external trophoblast and the more internal embryoblast (Fig. 4.4 B-8 and F-3). The trophoblast corresponds to the extraembryonic ectoderm, which is formed in the case of meroblastic development in monotremes and sauropsids (Blüm 1986, Smith 2015). The embryoblast (the actual embryo) consists of totipotent, embryonic stem cells and can form the source for creating chimaeras and transgenic animals in experimental biology (Houdebine 1997). A separation of the embryoblast can result in polyembryony (Newman and Patterson 1910).

4.7 Extraembryonic membranes

The vitelline (yolk) sac represents the ancestral extraembryonic membrane, which is plesiomorphically present in all vertebrates (Greven 2004). In mammals, the vitelline sac is initially formed by the enclosure of yolk or the hollow blastocyst by mesodermal cells from the embryonic disk (i.e., the hypoblast) (Fig. 4.5 B/D). A migration of entoderm cells forms a bilaminar yolk sac (Fig. 4.4 E-6 and 5G). The vitelline sac is slightly (Marsupialia) or largely (Placentalia) reduced in therian mammals and is filled with fluid (Figs. 4.5 F, I and 4.6 E–J). The anlage of the vitelline sac is strong evidence for the telolecithal origin of therian eggs (i.e., a large amount of yolk is concentrated at one pole) and that the yolk was secondarily lost. It is likely that the vitelline sac initially had a nutritional (Luckett 1975, Smith 2015) and a respiratory function (Houillon 1972). At the beginning of vitelline sac formation, a comprehensive circulatory system develops. As in most vertebrates, the first blood islands and blood vessels are formed on the surface of the vitelline sac or near the food resources. The vitelline sac is connected to the middle gut of the embryo via a yolk stalk (Greven 2004). Blood from the mesodermal vitelline veins (blood vessels of the umbilical cord) transport the blood to the heart of the embryo. Later on, the vitelline sac loses this initial function. However, it continues to have a functional circulation during yolk sac placentation. The vitelline sac serves for the

▸ **Fig. 4.5: Extraembryonic membranes.** Formation of the plect-/pleuramnion (A, B), schizamnion (C, D), and allantois (E, F). (G) Egg layers and formation of extraembryonic membranes in a chicken (*Gallus gallus*). (H) Extraembryonic membranes of the platypus, *Ornithorhynchus anatinus* (Monotremata). (I) Extraembryonic membranes of the golden mole (Chrysochloridae, Placentalia). A–F, after Houillon (1972); H, after Luckett (1975); I, after Kardong (2008).


A)
ectoderm
mesoderm
amniotic fold
ectoamnion
tropho-
blast
ecto-
chorion
yolk vacuole
entoderm
B)
amniotic
cavity
yolk sac
formation of pleuramnion
E)
exocoel
amnion
amniotic
cavity
allantoic
cavity
HENSEN'S
node
primitive
streak
yolk sac
chorion
C)
ectoamnion
ectoderm
mesoderm
amnion
entoderm
yolk vacuole
D)
amniotic
cavity
yolk sac
exocoel
mesoderm
chorion
trophoblast
formation of schizamnion
F)
uterus
epithel
allantoic cavity
embryo
amniotic cavity
exocoel
yolk sac
chorion
allantois formation
G)
cuticula
egg shell
outer membrane
inner membrane
exterior albumen
middle albumen
internal albumen
chalaza
vitelline membrane
amniotic
fold
exocoel
yolk
amnion
embryo
allantois
air
cell
exocoel
H)
shell membrane
egg shell
amnion-chorion-connection
amniotic cavity
allantois
yolk sac
exocoel
chorio-allantois-membrane
chorio-vitelline-membrane
I)
somatic (avascular) mesoderm
splanchnic (vascular) mesoderm
yolk sac
yolk sac (= chorio-
vitelline) placenta
(vascular)
ectoderm
endoderm
amniotic cavity
exocoel
allantoic
cavity
trophoblast
allantoic
(= chorioallantoic)
placenta
(vascular)

formation of the primary blood elements in haplorhine primates, in which it does not form a placenta (Carter 2015). As soon as it is almost fully reduced, hematopoiesis is transferred to the liver (Houillon 1972). The fusion of the vitelline sac and the chorion results in a choriovitelline membrane (yolk sac placenta) (Figs. 4.5 H–I and 4.6 E, G–J).

Characteristic for all amniotes, including monotremes and therian mammals, is the presence of three additional extraembryonic (fetal) membranes, which develop from the ventral side of the embryo's trunk: chorion (or serosa in sauropsids), amnion, and allantois. This condition is called cleidoic egg (from Greek Κλειστός, kleidos, meaning "enclosed").

The chorion develops from trophectoderm (synonym: trophoblast) and from extraembryonic mesoderm (Fig. 4.5 A–F and H–I), which is derived from the splanchno- and/or somatogenetic embryonic mesoderm (Fig. 4.4 C). The chorion is non-vascular and primarily responsible for gas exchange.

The amnion serves as a shock absorber and protects the embryo against desiccation (Gilbert 2006). It can form in two different ways: by folding (Fig. 4.5 A–B) or through the formation of a cavity (Fig. 4.5 C–D).

The folding process (plect- or pleuramnion) is most likely ancestral, and it is visible in sauropsids (Fig. 4.5G), monotremes, marsupials, lagomorphs, artio- and perissodactyls, all carnivorans, eulipotyphlans, and strepsirrhine primates (Fig. 4.5 A–B; Houillon 1972, Tyndale-Biscoe 2005). Initially, the embryoblast is situated on the top of the blastocyst. The trophoblast is formed below the embryoblast, namely around the yolk vacuole. The lower layer of the embryoblast is formed by entoderm (here called entophyll). The upper layer is made up of ectoderm and mesoderm and is embedded inside the upper part of the trophoblast (i.e., ectoamnion). The ectoamnion is ventrally connected to the rest of the trophoblast (i.e., ectochorion) (Fig. 4.5 A). Ectoamnion and ectochorion form dorsal folds (i.e., amniotic fold), which fuse and enclose the amniotic cavity (Fig. 4.5 B). Subsequently, the extraembryonic mesoderm expands inside the amniotic folds and an extraembryonic coelom (exocoel) appears.

In the pig, for example, amnion formation is finished when ten somite pairs (Fig. 4.10-4–5) are formed. At the base of the amniotic cavity, the embryo develops, and the formation of the primitive streak (Fig. 4.5 E) indicates the differentiation of the embryonic ectoderm (cf. Fig. 4.4 C-2).

Compared with marsupials, most erinaceids, soricids, tenrecids, chiropterans, and primates (including humans) show a full coverage of the embryoblast by the trophoblast (Fig. 4.5 C). The embryoblast remains compact for some time. Later on, vacuoles develop and fuse in the upper part of the embryoblast (ectoamnion) and other cell elements. Hence, a cleft is formed that expands to the amniotic cavity (Fig. 4.5 C–D; schizamnion). The roof of this cavity represents the ectoamnion, whereas the bottom is formed by the embryo proper. The mesoderm is more involved in amnion formation when compared with the pleuramnion and aligns early to the trophoblast (Fig. 4.5 D). The early formation of the amnion, which appears even before the formation of the primitive streak in humans, is associated with the comprehensive reduction of the yolk sac. This is also correlated to the size of the mesoderm. The yolk sac is surrounded by exocoel from its first appearance on the entoderm. The yolk sac does not take part in the formation of the inner wall of the trophoblast.

In most rodents, the embryoblast sinks into the yolk vacuole (like the clapper of a bell) but keeps its upper connection to the trophoblast. The trophoblast persists, thickens, and later fuses to the maternal epithelium. The yolk sac is well developed and aligns closely to the rest of the trophoblast, which is finally degenerated in this area. The formation of the amnion is a complex mechanism (ectochorial cyst formation), which can be derived from the schizamnion, namely from delamination of the embryoblast.

The allantois develops from extraembryonic entoderm and mesoderm (Fig. 4.5 E–F). It is vascularized and is responsible for the removal of nitrogenous waste. During development, the allantois expands and suppresses the exocoelomic cavity (Fig. 4.5 F). In monotremes, the allantois begins to form at the 27-cell stage and rapidly grows into the exocoel. It fuses with the chorion and forms an expanded respiratory surface. In monotremes, the allantois has a similar size as the vitelline sac (Figs. 4.3 A and 4.5 H) (Houillon 1972). In marsupials, the allantois is smaller (Fig. 4.6 G–J). In placental mammals (and some marsupials), it fuses with the chorion to form the chorioallantois (Fig. 4.5 I), an expanded organ for respiration (Fig. 4.5 H–I) (following the ground pattern of amniotes; Zeller 2004b). The chorioallantoic placenta is primarily responsible for gas exchange and calcium transport from the shell to the embryo (Greven 2004), but evolved further functions such as nutrient transfer, hormone secretion, and immune responses (Benirschke and Kaufmann 1995, Carter 2012).

Tab. 4.3: Comparison of selected life history features of different groups of placental mammals. Mainly summarized after Hayssen *et al.* (1993) and Wildman *et al.* (2006). Legend and other references within table.

Major taxon	Species	Common name	Placental interface	Placental shape	Maternofetal interdigitation	Gestation length	Litter size	Maturity level at birth	Eye opening after birth (0 = at birth)	Weaning	Neonatal mass	Neonatal size	Sexual maturity
Afrotheria													
Sirenia	*Dugong dugon*	Dugong	?	Zonary	Villous	~1 year	1 (2 rare)	Precocial	0	~1.5 year	20–35 kg	100–150 cm	9–10 years
Sirenia	*Trichechus manatus*	West Indian manatee	Endotheliochorial (1)	Zonary	Labyrinthine	~1 year	1 (2 rare)	Precocial	0	1–2 years	11–27 kg	100–120 cm	3–5 years
Hyracoidea	*Procavia capensis*	Rock hyrax	Hemochorial	Zonary	Labyrinthine	6–8 months	1 to 3	Precocial	0	3–7 months	140–405 g	190–204 mm	~15 months
Proboscidea	*Loxodonta africana*	African elephant	Endotheliochorial	Zonary	Labyrinthine	20–24 months	1 (2 rare)	Precocial	0	6–? Months	73–120 kg	?	7–15 years
Tubulidentata	*Orycteropus afer*	Aardvark	Endotheliochorial	Zonary	Labyrinthine	~7 months	1 (2 rare)	Naked with open eyes	0	?	1–2 kg	~550 mm	3 years
Afroinsectiphilia	*Elephantulus rufescens*	Elephant shrew	Hemochorial	Discoid	Labyrinthine	50–65 days	1 or 2	Precocial (not explicitly mobile)	0	15 days–1 month	9.3–13.0 g	?	~2 months
Chrysochloridae	*Amblysomus hottentotus*	Hottentot golden mole	Hemochorial	Discoid	Labyrinthine	?	1 or 2	Naked and blind, but adults also blind	n/a	?	~4.5 g	47 mm	?
Afrosoricida	*Potamogale velox*	Giant otter shrew	Endotheliochorial (2)	Discoid	Labyrinthine	?	1 or 2	Altricial	?	?	?	?	?
Afrosoricida	*Tenrec ecaudatus*	Tailless tenrec	Hemochorial	Discoid	Labyrinthine	53–64 days	1 to 4	Altricial	9–15 days	25–30 days	10–27.4 g	84–92 mm	~6 months
Xenarthra													
Cingulata	*Dasypus novemcinctus*	Nine-banded armadillo	Hemochorial	Discoid	Villous (6)	3–5 months delay, 4–4.5 months development	4	Altricial	?	4–5 months	28.6–133 g	25 cm	~1 year
Pilosa	*Myrmecophaga tridactyla*	Giant anteater	Hemochorial	Discoid	Villous (7–8)	~6 months	1	Precocial (not explicitly mobile)	0	3 months	1480–1720 g	?	?
Pilosa	*Choloepus didactylus*	Linnaeus's two-toed sloth	Hemochorial	Discoid	Trabecular	5.5–7 months	1	Precocial (not explicitly mobile)	0	5 months	364.3 g	16–18 cm	3–4.5 years

Tab. 4.3 (continued)

Major taxon	Species	Common name	Placental interface	Placental shape	Maternofetal interdigitation	Gestation length	Litter size	Maturity level at birth	Eye opening after birth (0 = at birth)	Weaning	Neonatal mass	Neonatal size	Sexual maturity
Euarchontoglires													
Primates	*Daubentonia madagascariensis*	Aye-aye	Epitheliochorial	Diffuse	Villous	?	1	?	?	6–7 months	?	?	?
Primates	*Homo sapiens*	Human	Hemochorial	Discoid	Villous	38 weeks	1 (2 rare)	Altricial	0	0.5–3 years	2500–4000 g	ca. 50 cm	12–14 years
Dermoptera	*Cynocephalus volans*	Philippine flying lemur	Hemochorial	Discoid	Labyrinthine	~150 days	1	?	?	~200 days	35.8 g	254 mm	?
Scandentia	*Tupaia glis*	Common tree shrew	Endotheliochorial	Bidiscoid	Labyrinthine	43–50 days	2 to 5	Altricial	13–25 days	~30 days	10–15 g	79–140 mm	3– 6 months
Rodentia	*Marmota monax*	Groundhog	Hemochorial	Discoid	Labyrinthine	30–40 days	4 to 9	Altricial	20–28 days	4–5 weeks	23.7–32.3 g	85–89 mm	~1 year
Rodentia	*Rattus norvegicus*	Brown rat	Hemochorial	Discoid	Labyrinthine	20–24 days	2 to 12	Altricial	5–22 days	4 weeks	5.27–7.35 g	52 mm	27–47 days
Rodentia	*Mus musculus*	House mouse	Hemochorial	Discoid	Labyrinthine	18–21 days	2 to 8	Altricial	12–15 days	3 weeks	0.8–1.14 g	22 mm	2 months
Rodentia	*Myocastor coypus*	Nutria	Hemochorial	Discoid	Labyrinthine	120–150 days	1 to 9 (up to 14 rare)	Precocial (not explicitly mobile)	0	5–10 weeks	100–250 g	?	2–9 months
Rodentia	*Hystrix africaeaustralis*	Cape porcupine	Hemochorial	Discoid	Labyrinthine	93–112 days	1 to 4	Precocial (not explicitly mobile)	0	~7 weeks	~300 g	?	8–18 months
Lagomorpha	*Oryctolagus cuniculus*	European rabbit	Hemochorial	Discoid	Labyrinthine	28–37 days	4 to 7	Altricial	10 days	22–25 days	30–45 g	117,70–119,07 mm	5–8 months
Lagomorpha	*Ochotona alpina*	Alpine pika	Hemochorial	Discoid	Labyrinthine	30 days	3 to 8	Altricial	8–10 days	20–22 days	6–10 g	?	25–30 days
Laurasiatheria													
Eulipotyphla	*Talpa europaea*	European Mole	Endotheliochorial	Discoid	Labyrinthine	30–40 days	2 to 6	Altricial	20–22 days	3–5 weeks	3–3.5 g	30–44 mm	~1 year
Eulipotyphla	*Erinaceus europaeus*	European hedgehog	Hemochorial	Discoid	Labyrinthine	5–6 weeks	2 to 8	Altricial	8–22 days	38–45 days	10.7–17.17 g	25–91.5 mm	10–12 months

Tab. 4.3 (continued)

Major taxon	Species	Common name	Placental interface	Placental shape	Maternofetal interdigitation	Gestation length	Litter size	Maturity level at birth	Eye opening after birth (0 = at birth)	Weaning	Neonatal mass	Neonatal size	Sexual maturity
Eulipotyphla	*Crocidura leucodon*	Bicolored white-toothed shrew	Hemochorial	Discoid	Labyrinthine	20–35 days	2 to 4	Altricial	9–13 days	16–26 days	0.8–1.0 g	?	3–4 months
Chiroptera	*Pteropus poliocephalus*	Grey-headed flying fox	Hemochorial (3)	Discoid	Labyrinthine	6 months	1	Altricial	1–10 days	?	70–90 g	60 mm	18 months
Chiroptera	*Megaderma lyra*	Greater false vampire bat	Hemochorial (4)	Discoid	Labyrinthine	150–160 days	1 (2 rare)	?	0	2 months	7–8 g	?	15–18 months
Chiroptera	*Molossus molossus*	Velvety free-tailed bat	Hemochorial	Discoid	Labyrinthine	~3.5 months	1	?	0	1.5–4 months	3.3–3.9 g	31.67–57.5 mm	3 months ?
Chiroptera	*Macrotus californicus*	California leaf-nosed bat	Hemochorial (5)	Discoid	Labyrinthine	8–9 months with about 4.5 months diapause	1	?	0	1 month	?	?	3–16 months
Pholidota	*Manis tricuspis*	Tree pangolin	Epitheliochorial	Diffuse	Villous	>5 months	1	Precocial (not explicitly mobile)	0–9 days	3–7.5 months	90–150 g	300–350 mm	2 years
Carnivora	*Vulpes vulpes*	Red fox	Endotheliochorial	Zonary	Lamellar	51–60 days	1 to 7	Altricial	8–14 days	5–10 weeks	50–150 g	131–150 mm	9–12 months
Carnivora	*Felis silvestris*	Wild cat	Endotheliochorial	Zonary	Lamellar	56–69 days	3–5 (up to 10 rare)	Altricial	1–15 days	3–4 months	82–149 g	126.7–155 mm	8–14 months
Perissodactyla	*Rhinoceros unicornis*	Indian rhinoceros	Epitheliochorial	Diffuse	Villous	463–488 days	1 (2 rare)	Precocial	0	12–18 months	33.75–71.3 kg	96.5–122 cm	4–7 years
Perissodactyla	*Tapirus* spp.	Tapir	Epitheliochorial	Diffuse	Villous	~13 months	1	Precocial	0	6–8 months	6–10.20 kg	72 cm	3–4.5 years
Perissodactyla	*Equus caballus*	Horse	Epitheliochorial	Diffuse	Villous	320–340 days	1	Precocial	0	6–12 months	?	?	2 years
Cetartiodactyla	*Lama glama*	Llama	Epitheliochorial	Diffuse	Villous	11–13 months	1	Precocial	0	4–6 months	8–16 kg	?	?
Cetartiodactyla	*Sus scrofa*	Domestic pig/ wild boar	Epitheliochorial	Diffuse	Folded	~4 months	4 to 12	Precocial	0	2.5–4 months	500–1534 g	24 cm	0.75–1.75 years
Cetartiodactyla	*Balaenoptera musculus*	Blue whale	Epitheliochorial	Diffuse	Villous	10–12 months	1	Precocial	0	6–7 months	2500 kg	700–800 cm	8–10 years

Tab. 4.3 (continued)

Major taxon	Species	Common name	Placental interface	Placental shape	Maternofetal interdigitation	Gestation length	Litter size	Maturity level at birth	Eye opening after birth (0 = at birth)	Weaning	Neonatal mass	Neonatal size	Sexual maturity
Cetartiodactyla	*Delphinus delphis*	Short-beaked common dolphin	Epitheliochorial	Diffuse	Villous	~11 months	1	Precocial	0	10 months	6.8–7.35 kg	75–90 cm	3–7 years
Cetartiodactyla	*Hippopotamus amphibius*	Hippopotamus	Epitheliochorial	Diffuse	Villous	~8 months	1 (2 very rare)	Precocial	0	9–14 months	25–50 kg	748–1270 mm	3–9 years
Cetartiodactyla	*Bos frontalis*	Gayal	Epitheliochorial	Cotyledonary	Villous	8–9 months	1 or 2	Precocial	0	4.5 months	13–33 kg	?	1–2 years

Placenta types, cited after Wildman *et al.* 2006, supplement.
Placental interface: The placental interface describes the degree of invasiveness of fetal (i.e., placental) tissue into maternal tissue, with epitheliochorial being least invasive and hemochorial being most invasive. There are six types of placental interface:
a. Epitheliochorial: the fetal chorion is in contact with the endometrium epithelium, and the blastocyst does not invade the maternal endometrium.
b. Syndesmochorial: the fetal chorion destroys the uterine epithelium; this character state was thought to exist in sheep and goats, but its existence has been refuted more recently (2).
c. Endotheliochorial: the fetal chorion erodes the endometrial epithelium and connective tissue, resulting in apposition to the uterine endothelium.
d. Hemochorial: the most deeply invasive form of placental interface. The trophoblast erodes all of the layers until the maternal vessels and the fetal trophoblast are directly in contact. This type of interface has been subdivided into three subtypes according to the number of layers of the cytotrophoblast: (i) hemomonochorial, the outer trophoblast layer is constructed of syncytiotrophoblast only; (ii) hemodichorial, only one side of the trophoblast is covered by cytotrophoblast; (iii) hemotrichorial, both sides of the trophoblast are covered by the cytotrophoblast.
Placental shape: The shape and area of the maternal side of the placenta where it interfaces with maternal tissue. There are five types of placental shape: (a) *Diffuse:* maternal-fetal interdigitation extends over the entire surface of the chorionic sac. (b) *Cotyledonary:* many spotlike areas of maternal-fetal interdigitation. (c) *Zonary:* ringlike area of maternal-fetal interdigitation. (d) *Bidiscoid:* two disklike areas of maternal-fetal interdigitation. (e) *Discoid:* a single disklike area of maternal-fetal interdigitation.
Maternofetal interdigitation: The form of contact between maternal and fetal tissues and/or blood. There are five types of maternofetal interdigitation: (a) *Folded:* ridgelike folds of the chorion that fit into grooves of the uterine mucosa. (b) *Lamellar:* ridgelike folds multiply in branch to form complicated columnlike folds. (c) *Trabecular:* branching folds in which leaflike and fingerlike villi branch off. (d) *Villous:* treelike branching of the chorion. (e) *Labyrinthine:* tissue block of trophoblast penetrated by weblike channels that are filled with either maternal blood or fetal capillaries.
Additional references: 1, Carter *et al.* (2008); 2, Carter *et al.* (2006); 3, Karim and Bhatnaga (1996); 4, Bhiwgade (1990); 5, Bodley (1974); 6, Enders (1960); 7, Becher (1931); 8, Mess *et al.* (2012).

4.8 Placentation

In monotremes, the vitelline sac is fused with the chorion (Figs. 4.3 A and 4.5 H; synonyms: choriovitelline, omphalopleura) and is able to resorb histotrophs, which are nutricial secretions from the mother's placenta, via its parietal (chorion) wall. One could hypothesize this form of nutrition to be an ancestral kind of placentation in amniotes because sauropsids also resorb nutrients via their vitelline sac (Mossman 1987, Zeller 2004b).

In some vertebrate taxa, viviparity is accomplished through the presence of a placenta, a specialized interface between maternal and fetal tissues. A true vitelline placenta is known from some viviparous squamates, in which the choriovitelline membrane is largely associated to the uterine epithelium (Greven 2004, Kardong 2008, Blackburn 2015). A close association of the chorion and the vitelline membrane seems to be the ancestral amniotic condition with the potential to evolve a true placenta as seen in viviparous squamates and possibly characterizing the ancestral condition for therian mammals. In other groups, viviparity is the result of retaining the egg in the body and only little or no maternal nutrition is provided, and the embryo is nourished by the yolk supplied in the egg. In some vertebrate species, embryonic consumption of maternal tissue, litter mates, or specialized eggs takes place. Viviparity evolved more than 100 times within squamates (Blackburn 2015). Most of those species rely on yolk nutrition and have some specialized fetal membranes for gas, water, and mineral exchange. All mammals and many squamates provide maternal nutrition in the form of uterine secretions, which is absorbed by extraembryonic or placental tissue (histotrophy), or by exchange of nutrients, waste products, and gas by maternal and fetal blood supplies (hemotrophy) (Smith 2015).

In therian mammals, matrotrophic viviparity involves maternal nutrition beyond the ancestral yolk supply (Mess and Carter 2006). This involves the formation of a placenta but results in a reduction of yolk content and litter size (Greven 2004). In amniotes, the chorion may fuse with the yolk sac (= vitelline membrane) to form the choriovitelline placenta (synonyms: yolk sac placenta, omphaloplacenta) and/or with the allantois to form the chorioallantoic placenta (Fig. 4.5 I). In monotremes and many squamates, choriovitelline and chorioallantoic membranes are well developed (Fig. 4.5 H). The first one serves for nutrition, the second one is responsible for gas exchange (respiration).

With the help of proteases, the trophoblast (chorioectoderm) of many therian mammals invades the endometrium, which is formed by the gland-rich uterine epithelium and the vascular connective tissue. As such, the trophoblast enables the implantation and embedding (nidation) of the embryoblast into the uterine wall and becomes a part of the embryonic aspect of the placenta (Greven 2004).

In addition to early nutrient exchange, this basic placenta type is important for the production of growth factors, binding proteins, and receptors, as well as for blood formation (i.e., hematopoiesis) and cholesterol production (Carter 2012).

Correlated with the small yolk sac, an association of the chorioallantoic membrane and the maternal epithelium (chorioallantoic placenta) is mainly found in placental mammals (Fig. 4.6 F). However, a wide range of both placenta types occurs among all clades of therian mammals (Smith 2015) (Figs. 4.5 I and 4.6 E–J, M). The chorioallantoic placenta is highly effective in that embryonic waste is extensively discharged.

Independent of the specific fetal membrane contribution to the placenta, different types, subtypes, variations, and homoplastic developments of invading the uterine lining exist (Smith 2015) (Tab. 4.3).

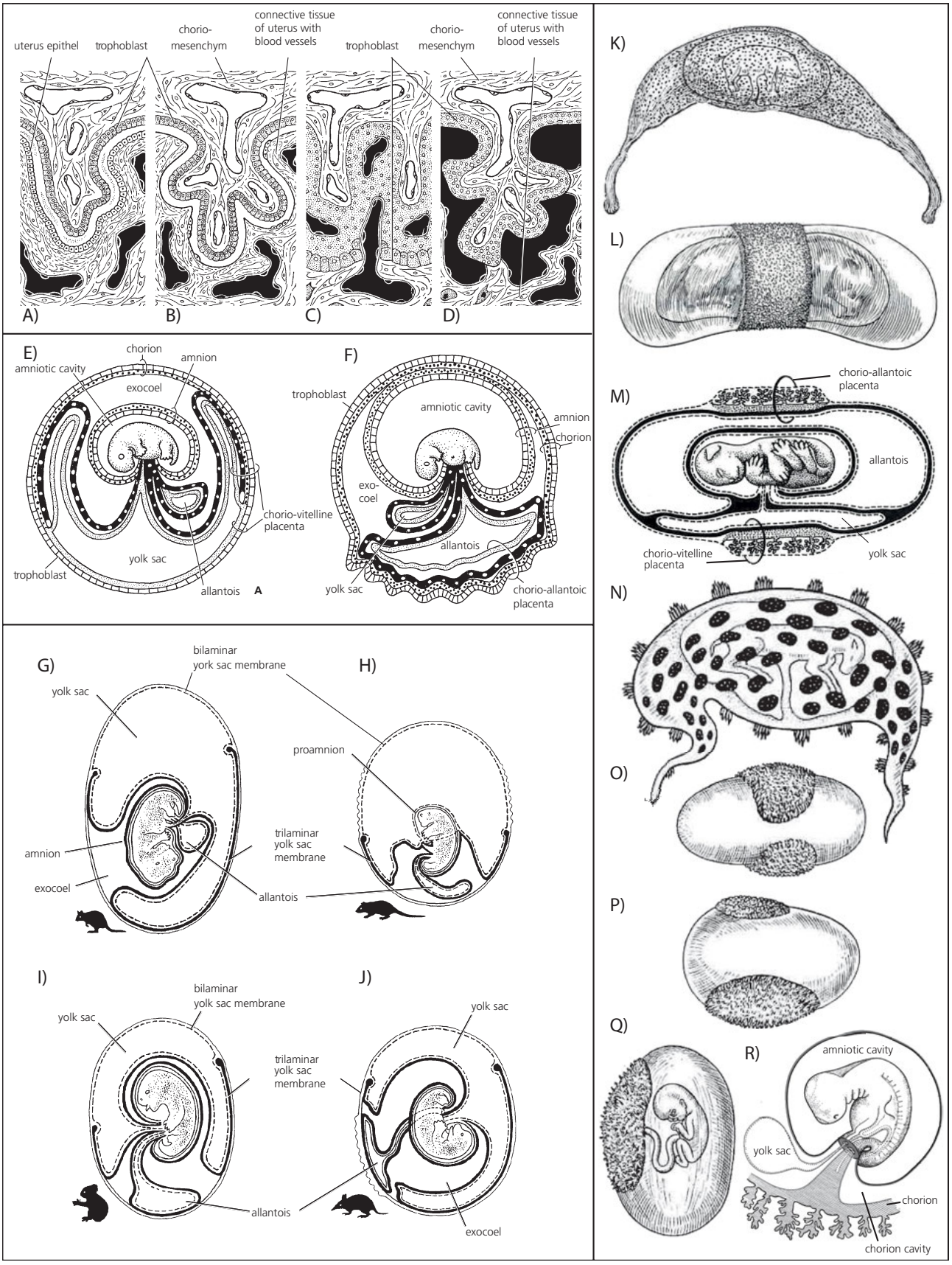
The least invasive type is the epitheliochorial placenta (in some cases it is not even invasive). Here the epithelia of the chorion and the uterus align to each other without invasion or erosion of uterine tissue. Nutrition is exchanged through the epithelia (Fig. 4.6 A). An invasive placenta type was certainly present in the ground pattern of placental mammals (Mess and Carter 2006, Elliot and Crespi 2009). The uterine epithelium can be partly destroyed, and the chorionic villi can be covered by epithelium and have a large contact surface to the connective tissue of the uterus (formerly known as syndesmochorial placenta). This type is restricted to small areas (cotyledonary or multiplex placenta) (Fig. 4.6 B).

The endotheliochorial placenta involves an invasion of extraembryonic tissue through the uterine lining and a direct contact of the maternal blood vessels with the endothelium (Fig. 4.6 C).

The hemochorial placenta involves the erosion of maternal blood vessels. This results in the accumulation of maternal blood around the chorioallantoic tissue (Fig. 4.6 D).

The ways in which extraembryonic and maternal tissue interdigitate and increase the surface of contact (villous, folded or lamellar trabecular, or labyrinthine) differ among taxa and homoplastic developments exist.

In some cases, multinucleate cells (syncytia) from mother and fetus can develop at their interface to increase the efficiency of nutritional exchange. Syncytia can


uterus epithel
trophoblast
chorio-mesenchym
connective tissue of uterus with blood vessels
trophoblast
chorio-mesenchym
connective tissue of uterus with blood vessels
A)
B)
C)
D)
E)
amniotic cavity
chorion
amnion
exocoel
chorio-vitelline placenta
yolk sac
trophoblast
allantois
A
F)
trophoblast
amniotic cavity
amnion
chorion
exo-coel
allantois
yolk sac
chorio-allantoic placenta
G)
bilaminar york sac membrane
yolk sac
amnion
exocoel
allantois
H)
proamnion
trilaminar yolk sac membrane
I)
bilaminar yolk sac membrane
yolk sac
allantois
J)
yolk sac
trilaminar yolk sac membrane
exocoel
K)
L)
M)
chorio-allantoic placenta
allantois
chorio-vitelline placenta
yolk sac
N)
O)
P)
Q)
R)
amniotic cavity
yolk sac
chorion
chorion cavity

develop on the maternal and/or the fetal side; however, in some groups (placental ruminants and marsupial peramelids), a syncytium of both tissues occurs (e.g., Wooding and Burton 2008). Different types of external anatomy of the placenta exist among placental mammals (Tab. 4.3, Fig. 4.6 K–Q).

The embryonic part of the epitheliochorial placenta (afterbirth, decidua) is delivered without bleeding (placenta indeciduata). Because of the close association to the maternal tissue of the uterus, the multiplex and the hemochorial placentas are delivered with bleeding (placenta deciduata) (Greven 2004).

Marsupial eggs are small and only yolk vacuoles (i.e., yolk particles in Fig. 4.4 A) are present (Fig. 4.4 A, Da, E). They serve as nutrition to the cleavage cells (Starck 1995). It appears that initially there is significant histotrophic nutrition in all marsupial groups. Highly efficient maternal uterine glands can be present to nourish the embryo (tammar wallaby, *Macropus eugenii*). Hemotrophic nutrition in marsupials as well as placentals is independent of the degree of invasiveness.

After resorption of the shell in marsupials (Fig. 4.4 Da-4), the vitelline sac expands and reaches the chorion to form the choriovitelline placenta (Greven 2004). The vitelline sac is only partly covered by mesoderm, so that the marsupial yolk sac wall is bilaminar in its lower half and trilaminar in its upper pole (Fig. 4.6 G–J; Tyndale-Biscoe and Renfree 1987, Tyndale-Biscoe 2005). In some cases, the allantois reaches the chorion and a second chorioallantoic placenta can also be present (bandicoot, *Perameles*) (Greven 2004, Smith 2015) (Fig. 4.6 J). The choriovitelline placenta forms the final functional placenta in most marsupials (Fig. 4.6 G–J). A chorioallantoic placenta can also appear transitionally in several marsupials and contributes to the final functional placenta in peramelid marsupials (Tyndale-Biscoe and Renfree 1987: fig. 7-17; Smith 2015). Among marsupials, the most common type is the large yolk sac placenta, in which the allantois does not reach the chorion (quokka, *Setonix brachyurus*) (Fig. 4.6 G). In another type, the allantois reaches the chorion but is later reduced and a yolk sac placenta is formed (eastern quoll, *Dasyurus viverrinus*) (Fig. 4.6 H). The allantois can also strongly attach to the chorion to form a large yolk sac placenta (koala, *Phascolarctos cinereus*) (Fig. 4.6 I). Finally, both an enlarged allantois and a yolk sac placenta can be present at the same time (long-nosed bandicoot, *Perameles nasuta*) (Fig. 4.6 J). Most marsupials have an epitheliochorial contact between fetal and maternal parts of the placenta, and no erosion of maternal tissue occurs (many macropodids) (Fig. 4.6 A). However, endotheliochorial invasion of extraembryonic tissue to the maternal blood cells and maternal-fetal syncytia are also known (Roberts and Breed 1994, Zeller and Freyer 2002) (Fig. 4.6 C).

In contrast to marsupials, the placenta develops very early in placental mammals. Before it is implanted in the uterine wall, the blastocyst hatches from the zona pellucida (Greven 2004). The trophoblast, which enables attachment of the embryoblast to the uterine wall, was hypothesized to be a unique feature in placental mammals (Lillegraven *et al.* 1987, Lillegraven 2004). However, most authors (e.g., Johnson and Selwood 1996, Selwood and Hickford 1999, Selwood and Johnson 2006, Smith 2015) highlighted that this external layer of the chorion membrane is homologous to the external layer of the chorion (trophectoderm) of non-placental amniotes. In many placentals (and also some marsupials), however, it appears to have a generally high potential to enable strong implantation of the embryo in the uterus by forming very complex chorionic villi.

As shown above, a great diversity of placenta types, forms of implantation, and fetal-maternal integration evolved among placental mammal clades (Tab. 4.3, Fig. 4.6 A–D and K–Q) (Wildman *et al.* 2006). In general, two major types in the constellation of characters of placenta morphology and life history traits can be identified (Lewitus and Soligo 2011, Smith 2015).

◂ **Fig. 4.6: Placentation.** (A–D) Placenta types among placental mammals. (A, B) epitheliochorial, (C) endotheliochorial, and (D) hemochorial placenta. (E) Yolk sac placenta (opossum, Marsupialia). (F) Chorioallantoic placenta (bush baby, *Galago*; Placentalia). (G, H) Placentation types among marsupials; most common type is the large yolk sac placenta, allantois does not reach the chorion (quokka, *Setonix brachyurus*) (G); allantois reaches the chorion but is later reduced, yolk sac placenta (eastern quoll, *Dasyurus viverrinus*) (H); allantois closely attaches to the chorion, large yolk sac placenta (koala, *Phascolarctos cinereus*) (I); enlarged allantois and yolk sac placenta (*Perameles nasuta*) (J). (K–Q) External shape of the placenta in different placental mammals; placenta diffusa (domestic pig, *Sus scrofa domestica*) (K); placenta zonaria (carnivoran) (L, M), two basic types of placental structures as seen in a transitional stage of an implanted domestic cat embryo (*Felis catus*). Yolk sac and chorioallantoic placenta are present. The latter grows outward and takes over the function of the earlier, primary yolk sac placenta (M); placenta cotyledonaria (cattle, *Bos primigenius taurus*) (N); incomplete zonary placenta (raccoon, *Procyon lotor*) (O); placenta bidiscoidalis (many primates) (P); placenta discoidalis (human, *Homo sapiens*) (Q). (R) Schematic diagram of a human embryo of about 10 mm in length and its relation to the extraembryonic membranes. A–D, after Westheide and Rieger (2010); E–F, after Kardong (2008); G–J, after Tyndale-Biscoe and Renfree (1987); K, L, N–R, after Starck (1965); M, after Pough *et al.* (2012).

One type is represented by an epitheliochorial placenta interface (Fig. 4.6 A), a diffuse placenta shape, and a villous to trabecular maternofetal interdigitation. The yolk sac is free. Placental mammals with such a placental anatomy are generally characterized by a long life span, precocial and large neonates, small litter size, long gestation, late weaning and maturity, small social group size, and high interbirth intervals (e.g., the brown greater galago *Otolemur crassicaudatus* and the horse *Equus caballus*).

Another type is represented by a hemochorial placenta interface (Fig. 4.6 D), a discoid placenta shape (Fig. 4.6 Q), and a labyrinthine maternofetal interdigitation. The yolk sac is inverted. These placental mammals are generally characterized by a short life span, altricial and small neonates, large litter size, short gestation, early weaning and maturity, large social group size, and low interbirth interval (e.g., the rabbit *Oryctolagus cuniculus*; Lewitus and Soligo 2011). There are some obvious exceptions, such as humans who have the latter placenta shape (Martin 2007).

The chorioectoderm (trophoblast) was thought to be a unique feature in placental mammals, and to enable a particularly efficient immunological barrier in placental mammals. For that reason, placental mammalian fetuses would be longer retained in the womb and would reach a more advanced anatomical state at birth than marsupial mammals do (e.g., Lillegraven *et al*. 1987, Lillegraven 2004). However, more recently, several authors highlighted that the chorioectoderm is homologous among amniotes (see Smith 2015). Apparently, in most placental mammals, it has a particularly strong ability to invade the maternal tissue and to form complex integrations of the embryo/fetus and the mother. However, complex placentas are also found in some marsupials, which possibly evolved the ability of trophoblast invasion secondarily.

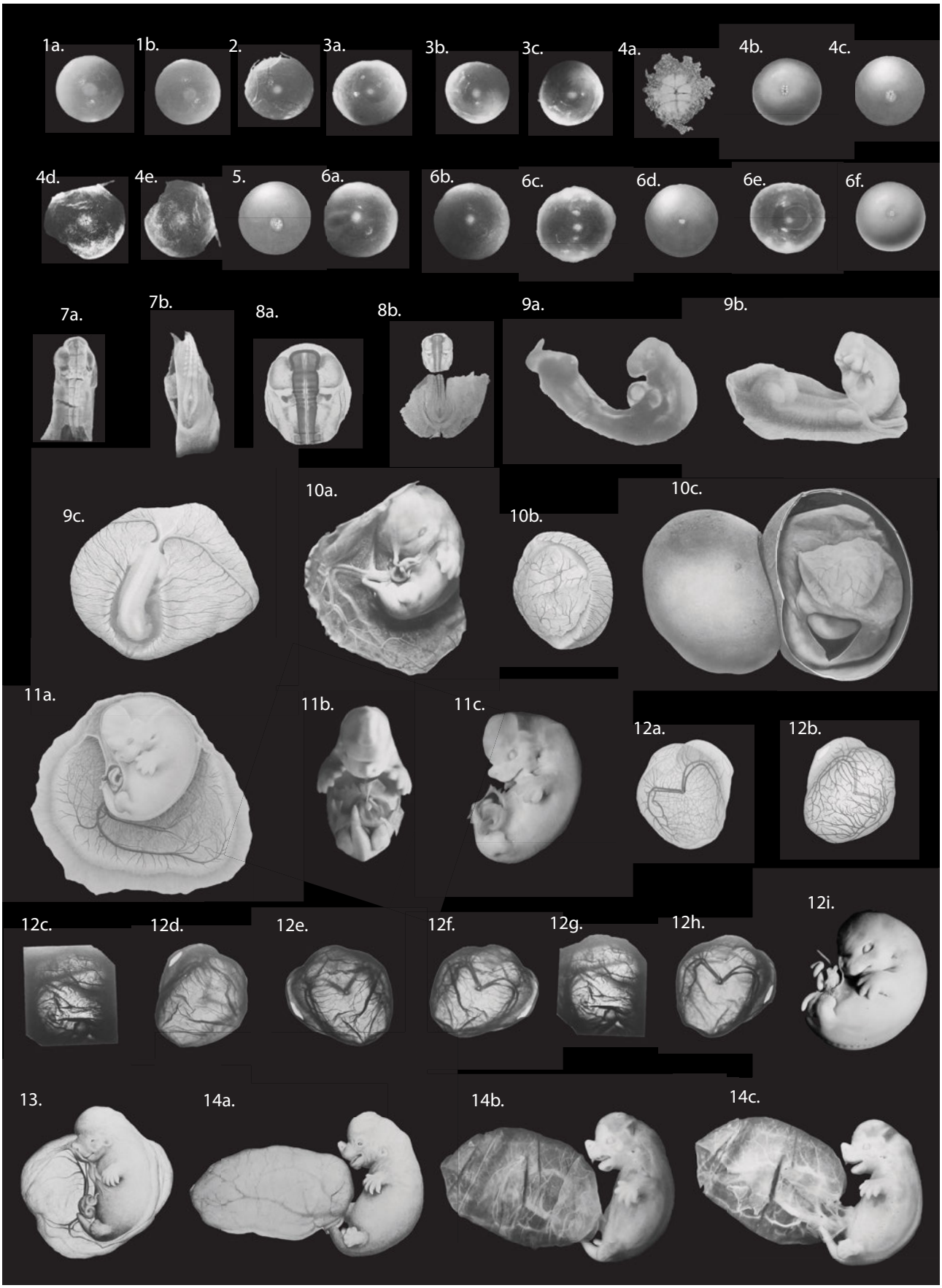
It is worth noting that the placental mammal embryo already invades the maternal tissue at a very early stage of development, whereas in marsupials implantation first appears at the beginning of the last trimester of intrauterine development. This means that marsupials, in general, have less time to build a complex placenta. No data are available on whether marsupial species with complex placentas show a relatively earlier implantation than species with less complex placentas. If so, not the immunological barrier but more strikingly the degree of embryonic nourishment enabled by the deep integration of maternal and fetal tissue could be a reason for the longer intrauterine development of placental mammals.

The chorioallantoic placenta, which is particularly, but not exclusively, present in placental mammals was thought to be highly effective for embryonic/fetal development. The integration of the allantois to the fetal part of the placenta suggests a highly effective removal of embryonic nitrogenic waste via the maternal circulatory system (Storch and Schröpfer 2004). It has been suggested that the choriovitelline placenta in marsupial mammals and other viviparous amniotes is less effective for waste removal because the allantois is largely or completely separated from the fetal/embryonic part of the placenta.

4.9 Evolution of organogenesis

After gastrulation, organ systems develop and differentiate (Fig. 4.4 C), including the skeletal, muscular, circulatory, digestive, reproductive, and neural systems (Keibel 1897, Starck 1965). The major mammalian groups only differ

▸ **Fig. 4.7: Embryonic series of *Ornithorhynchus anatinus* (Monotremata, Ornithorhynchidae) (Part 1/2).** Previously unpublished drawings originating from the Embryological collection of the Museum für Naturkunde Berlin, Germany. The drawings are part of the extensive collection of James Peter Hill (1873–1954). The origins of the drawings remain unknown, although Hill is known to have collected and studied *O. anatinus* specimens with colleagues at the end of the 19th century and the beginning of the 20th century (Watson 1955). A number of these drawings were previously published. Previously published images are cited below. We scanned the original drawings from the J.P. Hill Collection. (1a, b) M15 (unpublished), egg after removal of shell, 4.3 × 4.2 mm; (2) M16 (unpublished), egg after removal of shell; (3a, b, c) M17 (unpublished), egg after removal of shell, 5 × 4.5 mm; (4a) M18 (published as fig. 1 in Hughes 1993), 8-celled egg, 4-mm diameter; (4b, c, d, e) M18 (unpublished), 8-celled egg, 4-mm diameter; (5) M19 (unpublished), egg; (6a, b, c, d, e, f) M22 (unpublished), egg after removal of shell, 4.2 mm, blastodisk, 0.36 × 0.31 mm; (7a, b) M31 (unpublished), early embryo?; (8a) M32 (unpublished), cephalic region; (8b) M32 (unpublished), early embryo?; (9a) M43 (unpublished), embryo, TL about 6.5 mm; (9b) M43 (published as fig. 3 in Hughes and Hall 1998), embryo, TL about 6.5 mm, drawn by A. Cronin; (9c) M43 (unpublished), embryo, dorsal view, egg 17 × 14.5 mm, drawn by A. Cronin; (10a) M37 (unpublished), embryo; (10b) M37 (unpublished), allantois, 16.5 × 14 mm; (10c) M37 (unpublished), two eggs, egg size, 16.5 × 14 mm and 18 × 14 mm, embryo size 8 mm and 8.5 mm, respectively; (11a, b, c) M38 (unpublished), embryo; (12a, b, c, d, e, f, g, h, i) M39 (unpublished), embryo, egg size 16.5 × 15 mm; (13) M41 (unpublished), embryo, documented by G. Wilson; (14a) M40 (unpublished), embryo, TL: 9 mm, TL of egg: 15 mm, documented by G. Wilson, 1898; (14b) M40 (published as fig. 6 in Hughes and Hall 1998 and as fig. 12 in Hughes 1993), embryo, TL 9 mm, TL of egg 15 mm, documented by G. Wilson, 1898; (14c) M40 (published as fig. 16 in Hughes 1984), embryo, TL 9 mm, TL of egg 15 mm, documented by G. Wilson, 1898. This figure is continued in Fig. 4.8.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slightly in their general anatomical construction, and mainly proportional developmental changes distinguish the different clades. In the following, we only concentrate on external organ development due to comprehensive data availability.

4.9.1 Methodological framework

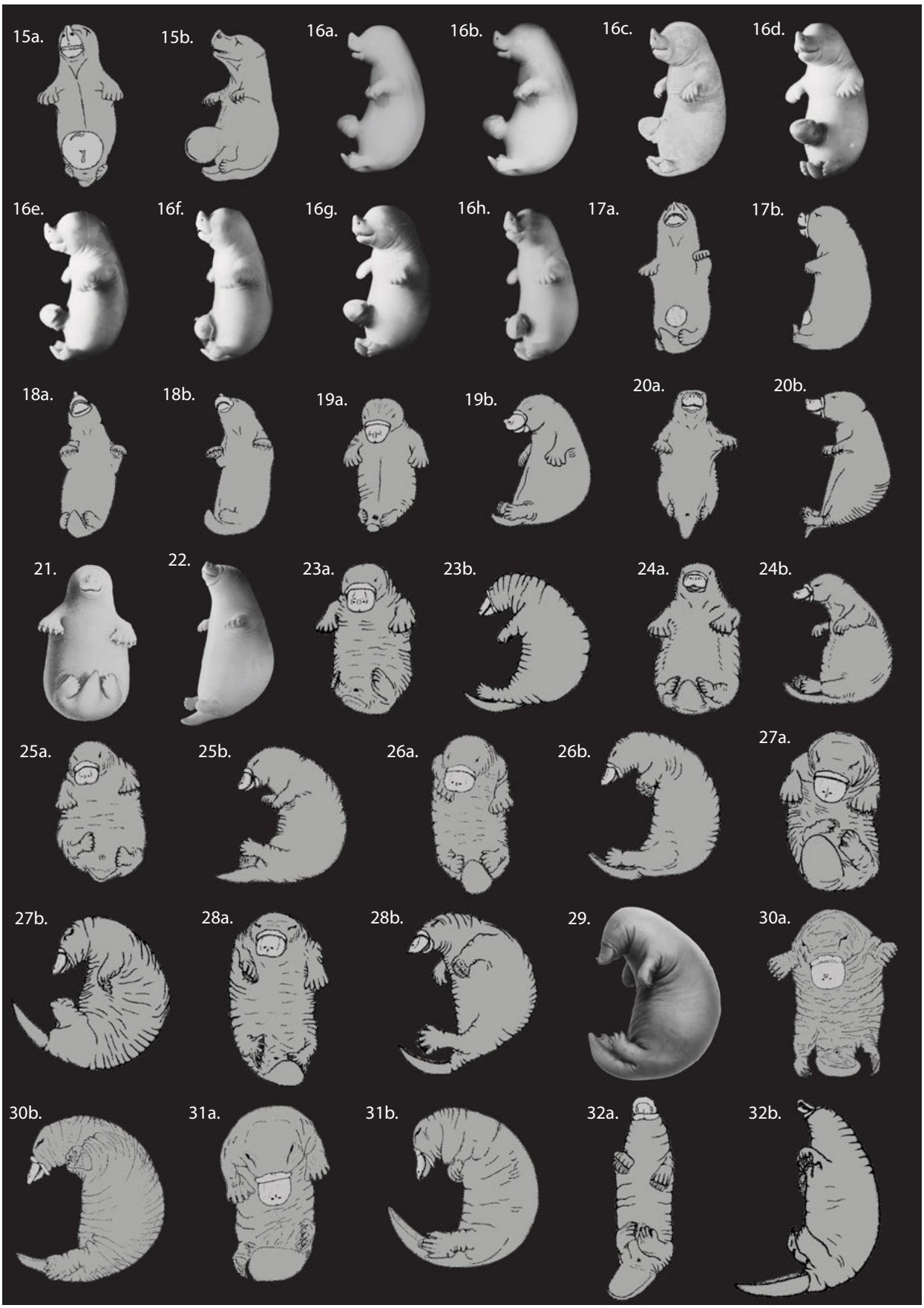
External characteristics are initially used to stage embryos in the progress of their development before investigating internal structures. External anatomy gives an initial impression of how far organs could be developed underneath the covering skin. For example, a paddlelike limb will not yet have fully developed phalanges and the eye anlage will not yet be able to process visual information. In recent years, developmental characters were the subject of several evolutionary studies in amniotes (e.g., Bininda-Emonds *et al.* 2002, 2003), mainly using two different methods. On the one hand, specific larval characters of lissamphibians were compared in a traditional cladistic sense (e.g., Haas 2003). On the other hand, new evolutionary approaches were developed on how to quantify the relative timing of ontogenetic characters through ontogeny in different species (e.g., Smith 1997, Jeffery *et al.* 2005, Harrison and Larsson 2008, Germain and Laurin 2009). Both approaches help to reconstruct ancestral ontogenies throughout evolutionary history. In the following, we use the second approach and provide new data in reconstructing the ancestral timing of organogenesis in the last common ancestor of placental mammals.

4.9.2 Historical background

In order to perform comparative studies on embryonic development, it is necessary to work with a comparable and consistent anatomical nomenclature. The first systematic scientific documentation of external embryonic characteristics dates back to von Soemmerring (1799), who documented the external anatomical development of 18 aborted human embryos ordered after the last menstrual cycles of their mothers (Hopwood 2007). The figures represented an idealistic illustration rather than actual specimens. During the 19th century, more comprehensive embryonic studies were provided. Franz Keibel edited a 16-volume series from 1897 to 1938, called "Normentafeln zur Entwicklungsgeschichte der Wirbeltiere" (normal plates of vertebrate embryonic development), on a variety of vertebrate embryos, including several mammalian species. He introduced his series with the development of the domestic pig (Fig. 4.11) (Keibel 1897). In this series, the author developed the approach of normal plates ("Normentafeln"), which uses a combination of illustrations and tables to describe the anatomy of individual embryos separately. In these, non-aborted embryos, without malformations (hence "normal"), were described and depicted. Although an enormous data set was provided by the authors of this book series and a conceptual framework was available in the form of evolutionary theory, the methodology was not yet developed to analyze different patterns of embryology quantitatively. Today, this can be done using new comparative methods that take into account phylogeny and shape changes (e.g., Richardson and Keuck 2002, Schmidt and Starck 2004, Maxwell and Harrison 2009). To this end, the relative or absolute timing of developmental characters is compared among species and ancestral developmental sequences are reconstructed (see below).

During the 20th century, embryological research developed more toward an experimental science, and important contributions to our understanding of embryonic mechanisms emerged (Hoßfeld and Olsson 2003b). A particular interest for experimental model organisms developed. For these species (e.g., chicken, mouse, fruit fly, and round worm), comprehensive staging tables were

▸ **Fig. 4.8: Embryonic series of *Ornithorhynchus anatinus* (Monotremata, Ornithorhynchidae) (Part 2/2).** Continuation of Fig. 4.7. (15a, b) NMA (National Museum of Australia, Canberra, Australia) 684 (modified from Manger *et al.* 1998: fig. 1), hatchling, CR length 13.5 mm; (16a, b) M44 (unpublished), hatchling, TL (total length) 16 mm; (16c) M44 (published as fig. 7 in Hughes and Hall 1998), hatchling, TL 16 mm; (16d, e, f, g, h) M44 (unpublished), hatchling, TL 16 mm; (17a, b) NMA 685 (modified from Manger *et al.* 1998: fig. 1), hatchling, CRL (crown-rump-length) 16.2 mm; (18a, b) NMA 686 (modified from Manger *et al.* 1998: fig. 1), hatchling, CRL 21.1 mm; (19a, b) NMA 687 (modified from Manger *et al.* 1998: fig. 1), hatchling, CRL 26.1 mm; (20a, b) NMA 689 (modified from Manger *et al.* 1998: fig. 2), hatchling, CRL 31.6 mm; (21) M42 (unpublished), young, documented by G. Wilson, 1898; (22) M45 (unpublished), young, TL 33 mm; (23a, b) NMA 690 (modified from Manger *et al.* 1998: fig. 2), young, CRL 37.6 mm; (24a, b) NMA 691 (modified from Manger *et al.* 1998: fig. 2), young, CRL 43.2 mm; (25a, b) NMA 692 (modified from Manger *et al.* 1998: fig. 3), young, CRL 56.6 mm; (26a, b) NMA 694 (modified from Manger *et al.* 1998: fig. 3), young, CRL 68.0 mm; (27a, b) NMA 695 (modified from Manger *et al.* 1998: fig. 3), young, CRL 68.3 mm; (28a, b) NMA 696 (modified from Manger *et al.* 1998: fig. 4), young, CRL 70.9 mm; (29) M- (unpublished), young, TL 80 mm; (30a, b) NMA 697 (modified from Manger *et al.* 1998: fig. 4), young, CRL 92.2 mm; (31a, b) MVM (Museum of Victoria, Melbourne, Australia) C27576 (modified from Manger *et al.* 1998: fig. 4), young, CRL 101.3 mm; (32a, b) NMA 698 (modified from Manger *et al.* 1998: fig. 5), young, CRL 150.0 mm.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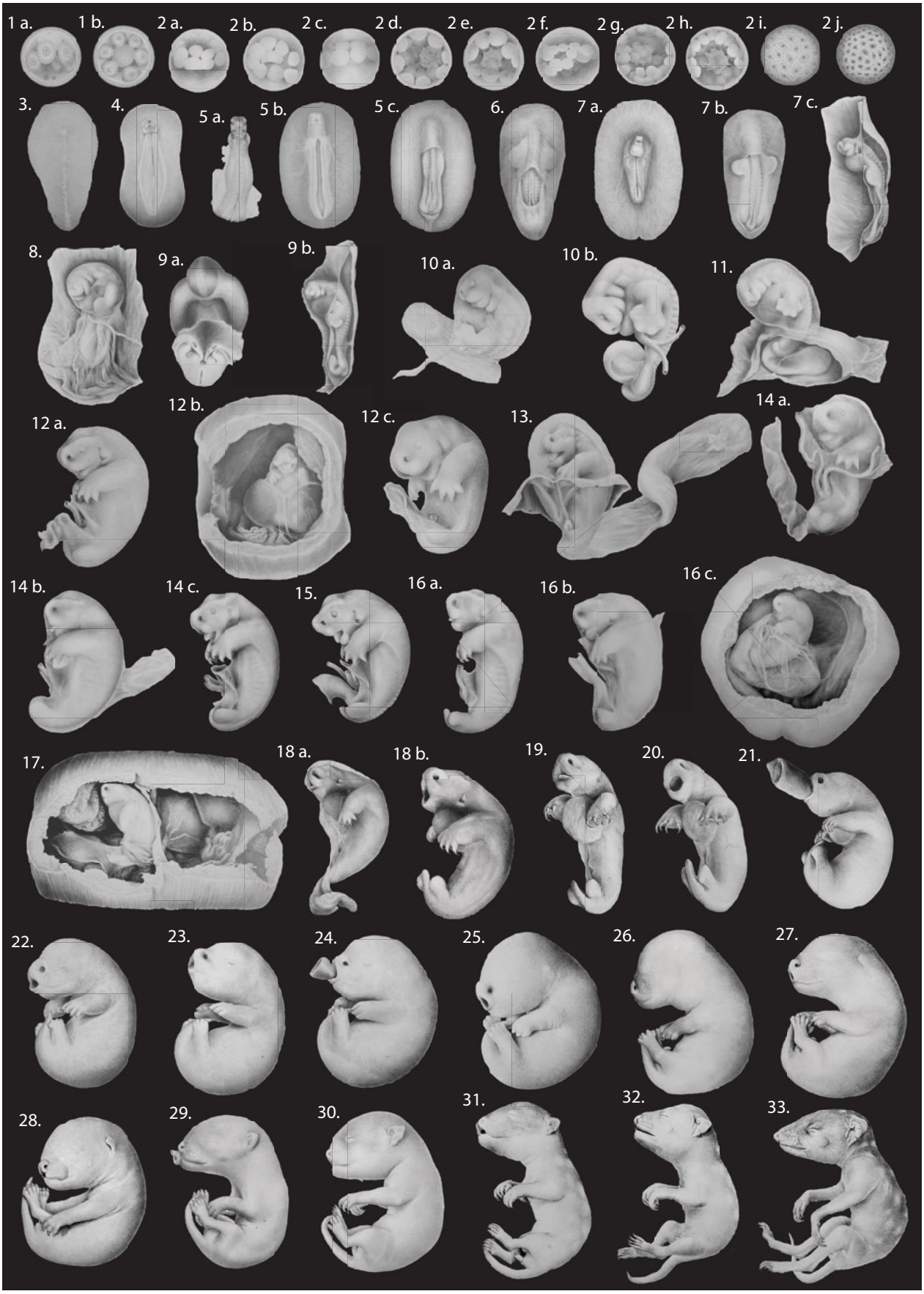
developed (Hamburger and Hamilton 1951, Harrison 1969, Theiler 1989). Based on a large number of embryonic specimens, artificially delimited stages of development, which illustrate the most common embryonic features at a defined period, were described and depicted. Intraspecific variability was neglected by presenting the most common morphotype. As such, embryonic stages do not represent real embryos but idealistic schemes for initial orientation in experimental biology.

4.9.3 Standard event system and heterochrony

The above-mentioned staging tables were created for particular species only and are difficult to compare with other, even closely related, species, making comparisons complicated. To resolve this problem, Werneburg (2009) developed the "Standard-Event-System" (SES) to study vertebrate embryos comparatively. An initial set of 104 discrete embryonic characters was defined that one can easily recognize in any vertebrate species independent of the age of the individual specimen or its taxonomic identity. In the following years, several species were described using the SES system and the count of characters has increased to 166 (updated data set: https://en.wikipedia.org/wiki/Standard_Event_System).

The relative appearance of particular organs and tissues differs among and within all major vertebrate groups. Heterochronic effects, evolutionary changes in the timing of developmental characters, appear that are characteristic for particular clades. In general, the earlier an organ appears in development, the longer it can grow, suppress, and relocate the expansion of other organs (Werneburg *et al.* 2015). This has been called the heterotopic effect (Zelditch and Fink 1996, McNamara 2002).

▸ **Fig. 4.9: Embryonic series of the eastern quoll *Dasyurus viverrinus*** (Marsupialia, Dasyuromorpha) based on drawings from the Embryological collection of the Museum für Naturkunde Berlin, Germany. The drawings are part of the extensive collection of James Peter Hill (1873–1954). To our knowledge, the drawings represent specimens from the captive breeding population of the eastern quoll *Dasyurus viverrinus* that Hill kept between 1899–1901 and 1904 (Watson 1955). A number of these drawings were previously published. We scanned the original drawings from the J.P. Hill Collection. (1a) Ms78 (published as fig. 53 on plate 6 in Hill 1910), 6-celled egg, 0.34-mm diameter, side view; (1b) Ms78 (published as fig. 54 on plate 6 in Hill 1910), 6-celled egg, 0.34-mm diameter, lower pole view; (2a) MS79 (published as fig. 57 on plate 6 in Hill 1910), 12-celled egg, 0.38-mm diameter, side view; (2b) MS79 (published as fig. 58 on plate 6 in Hill 1910), 12-celled egg, 0.38-mm diameter, lower pole view; (2c) MS79 (published as fig. 55 on plate 6 in Hill 1910), 16-celled egg, 0.37-mm diameter, side view; (2d) MS79 (published as fig. 56 on plate 6 in Hill 1910), 16-celled egg, 0.37-mm diameter, lower pole view; (2e) MS 79 (unpublished), 16-celled egg, 0.36-mm diameter; (2f) MS79 (published as fig. 60 on plate 6 in Hill 1910), 31-celled egg, 0.375-mm diameter, side view; (2g) MS79 (published as fig. 59 on plate 6 in Hill 1910), 31-celled egg, 0.375-mm diameter, lower pole view; (2h) MS 79 (unpublished), 32-celled egg, 0.375-mm diameter; (2i) MS79 (published as fig. 61 on plate 6 in Hill 1910) blastocyst, 0.39-mm diameter; (2j) MS79 (published as fig. 62 on plate 6 in Hill 1910) blastocyst, 0.4-mm diameter; (3) MS109 (unpublished), blastula 5.5-mm diameter; (4) MS115 (unpublished), 7.5-mm diameter; (5a) MS118 (unpublished), 15 somites, TL 6.37 mm; (5b) MS118 (unpublished); (5c) MS118 (unpublished), about 11 somites; (6) MS119 (unpublished); (7a) MS120 (unpublished); (7b) MS120 (unpublished); (7c) MS120 (unpublished), TL? 4.7 mm; (8) MS123 (unpublished, TL 4 mm; (9a) MS124 (unpublished); (9b) MS124 (unpublished), TL 4.8 mm; (10a) MS125 (unpublished), TL 3.7 mm; (10b) MS125 (unpublished), TL 3.6 mm; (11) MS126 (unpublished), TL 3.5 mm; (12a) MS127 (unpublished), TL 5.16 mm; (12b) MS127 (unpublished); (12c) MS127 (unpublished), TL 4.2 mm; (13) MS128 (unpublished), TL 5 mm; (14a) MS129 (unpublished), TL 5.4 mm; (14b) MS129 (unpublished), TL 5.5 mm; (14c) MS129 (unpublished), TL 5.4 mm; (15) MS194 (unpublished), TL 5 mm; (16a) MS131 (unpublished), TL 6 mm; (16b) MS131 (unpublished), TL 5.8 mm; (16c) MS131 (unpublished), TL 6 mm; (17) MS132 (unpublished), TL 6.5 mm; (18a) MS193 (published as fig. 1 on plate 1 in Hill and Osman-Hill 1955) embryo shortly before birth, TL 5.5 mm; (18b) MS193 (published as fig. 3 on plate 1 in Hill and Osman-Hill 1955 and as fig. 32B in Klima 1987) pouch young several hours old/neonate?, TL 6/5.5 mm?; (19) MS135 (published as fig. 2 on plate 1 in Hill and Osman-Hill 1955 and as fig. 32A in Klima 1987) neonate, TL 5.6 mm; (20) MS138 (published as fig. 4 on plate 1 in Hill and Osman-Hill 1955) neonate, 3 hours old, TL 6 mm; (21) MS 142 (published as fig. 5 on plate 1 in Hill and Osman-Hill 1955) pouch young attached to teat, 26 hours old, TL 6 mm; (22) MS148 (published as fig. 6 on plate 1 in Hill and Osman-Hill 1955) pouch young, about 3 days old, TL 7 mm; (23) MS149 (published as fig. 7 on plate 1 in Hill and Osman-Hill 1955) pouch young, 5–6 days old, TL 8 mm; (24) MS154 (published as fig. 8 on plate 1 in Hill and Osman-Hill 1955) pouch young attached to teat, about 7 days old, TL 8.5–9 mm; (25) MS158 (published as fig. 9 on plate 1 in Hill and Osman-Hill 1955) pouch young, about 10 days old, TL 10 mm; (26) MS164 (published as fig. 10 on plate 2 in Hill and Osman-Hill 1955) pouch young, about 14 days old, TL 13.5 mm; (27) MS173 (published as fig. 11 on plate 2 in Hill and Osman-Hill 1955 and as fig. 32C in Klima 1987) pouch young, 19 days old, TL 16.5 mm; (28) MS176 (published as fig. 12 on plate 2 in Hill and Osman-Hill 1955) pouch young, about 25 days old, TL 20 mm; (29) MS- (published as fig. 13 on plate 2 in Hill and Osman-Hill 1955) pouch young, about 35 days old, TL 24 mm; (30) MS179 (published as fig. 14 on plate 2 in Hill and Osman-Hill 1955) pouch young, about 41 old, TL 29 mm; (31) MS181 (published as fig. 15 on plate 2 in Hill and Osman-Hill 1955) pouch young, 46 days old, TL 42 mm; (32) MS182 (published as fig. 16 on plate 2 in Hill and Osman-Hill 1955) pouch young, just over 2 months old, TL 59 mm; (33) MS183 (published as fig. 17 on plate 2 in Hill and Osman-Hill 1955) pouch young, about 2.5 months old, TL 65 mm.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4.9.4 Data collection and evaluation

In a recent study (Werneburg *et al.* 2016), embryonic series of about 80 mammalian and non-mammalian tetrapod species were compared, and the relative timing of 166 external developmental characters (SES characters) was documented. All species in the study exhibit a different length of embryonic development with a gestation ranging from a few days (marsupials) to about 2 years (elephants). To make these developmental series comparable, development was scaled between 0 and 1 (Germain and Laurin 2009). Fertilization was defined as "0" and birth/hatching as "1". This means that each SES character was assigned a score between 0 and 1. These scores were mapped onto a molecular and evolutionary time scaled phylogeny. Using squared-change parsimony methodology (Felsenstein 1985), ancestral values were reconstructed for these continuous characters. The length of each branch in the phylogeny was considered for the calculation of the ancestral value, which finally represents a weighted mean of two values. Using this approach, the ancestral values were reconstructed for all characters of all nodes within the phylogeny.

4.9.5 Evolutionary patterns

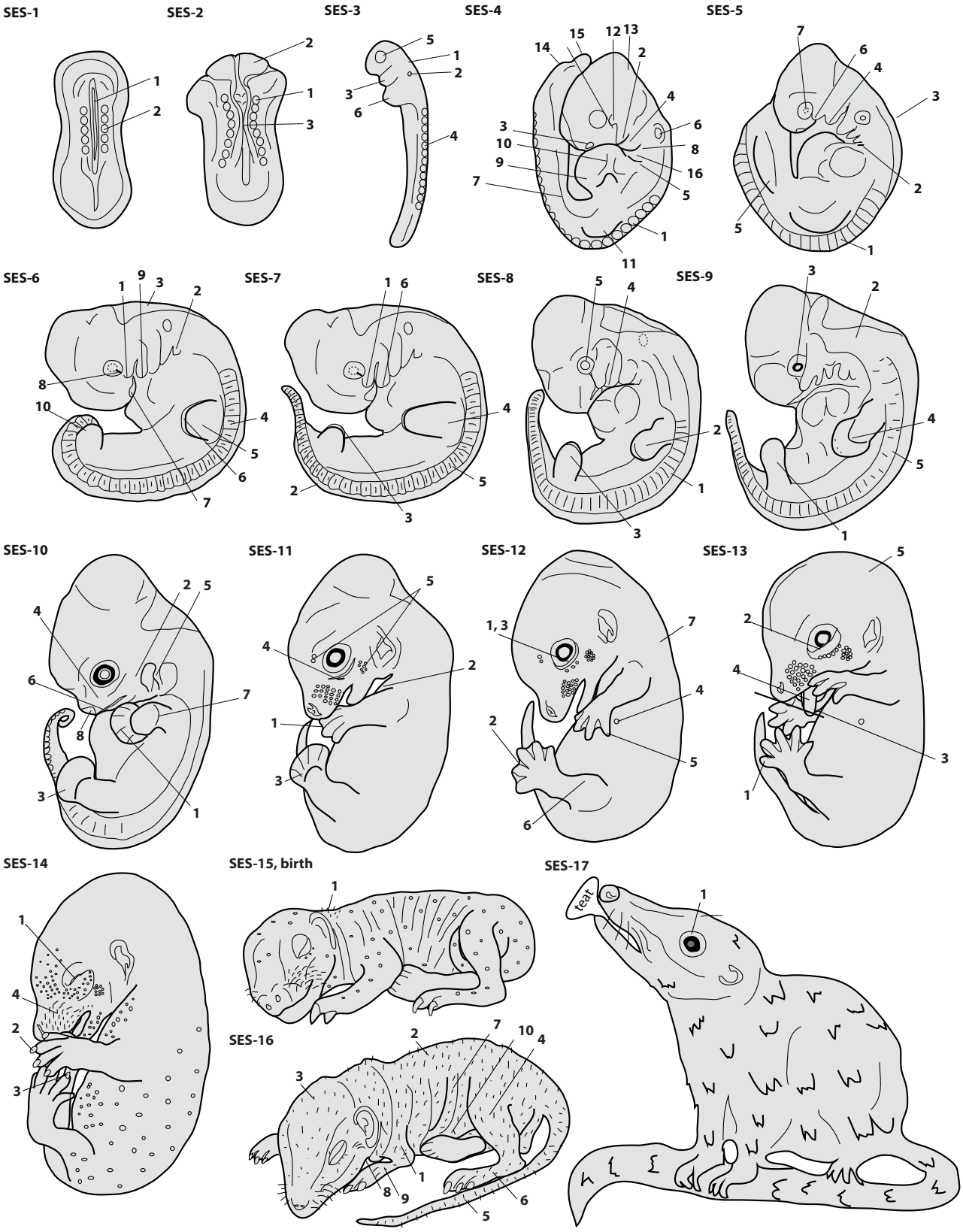
The major mammalian clades, Monotremata, Marsupialia, and Placentalia, differ in the relative reconstructed timing of various characters. These heterochronies can be interpreted by the different body proportions that the adults show – the earlier a character appears in ontogeny, the larger or more differentiated it is in the adult (Werneburg *et al.* 2015). Major differences occur around the time of birth/hatching, which can be interpreted by the specialized adaptations that the different life histories of the major mammalian groups require (see below and Werneburg and Geiger 2017).

The ancestral patterns of monotreme and marsupial organogenesis do not differ drastically from the developmental sequences known for the extant species (Werneburg *et al.* 2016). Therefore, we present here one representative embryonic series for each of these two clades, which include images never published before. Although the monotreme *Tachyglossus aculeatus*, the short-beaked echidna, is well documented in its external embryonic anatomy (e.g., Fig. 4.3 A, E; Semon 1894a–d; Werneburg and Sánchez-Villagra 2011), only little is known about the prehatching organogenesis of the platypus, *Ornithorhynchus anatinus* (Hughes and Hall 1988), because the eggs are incubated in breeding burrows, which are up to 30 m in length and difficult to access (Grant 1989). To illustrate marsupial development, we selected the eastern quoll, *Dasyurus viverrinus*. Only part of its embryonic anatomy was documented so far (e.g., Hill 1910, Hill and Osman-Hill 1955). We collected all available images for the platypus (Figs. 4.7–8) and eastern quoll (Fig. 4.9) housed in the collection of James P. Hill, which is now part of the Embryological Collection at the Museum für Naturkunde in Berlin, Germany. We ordered the specimens by the progress of development (following Werneburg 2009).

4.9.6 Embryology of the last common ancestor of Placentalia

In addition to monotremes and marsupials, we were particularly interested in the ancestral organogenesis of the last common ancestor of Placentalia because a variety of body shapes evolved within this clade, including diverse terrestrial, flying, aquatic, fossorial, and arboreal forms. This pattern is reflected in the diversity of embryonic shapes, different life history modes, and anatomical conditions at birth. We present the reconstructed timing of developmental characters for the placental mammal ancestor and created a staging table for this ancestor (Tab. 4.4). When a new state of a character complex such as limb development appeared in the developmental sequence, a new SES stage was assigned. We were able to create 18 SES-stages. Finally, the character sequence was the basis for a graphical reconstruction. For illustration, we mostly relied on the staging system available for the tree shrew, *Tupaia javanica* (de Lange and Nierstrasz 1932), because the adults of this species look similar to the last common ancestor of placentals reconstructed by O'Leary *et al.* (2013). We modified the external anatomy of these embryos based on our phylogenetic reconstruction for

▸ **Fig. 4.10: Schematic illustration of organ development in the last common ancestor of Placentalia.** For general orientation of body proportion, the embryogenesis of the tree shrew *Tupaia javanica* was used (de Lange and Nierstrasz 1932), with the exception of SES stage 4 (modified after a comparable stage of the house mouse *Mus musculus;* Theiler 1989) and SES stage 17 (after the reconstruction of the adult ancestral placental mammal; O'Leary *et al.* 2013). The original drawings were modified to illustrate the sequence of embryonic character appearance in the last common ancestor of placental mammals. Numbers in each picture refer to the fine grade sequence of character appearance in each stage. SES stage 18, weaning, is not illustrated. Embryos not to scale (see Tab. 4.4).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SES-17
tear

Tab. 4.4: Embryonic development of the last common ancestor of Placentalia as reconstructed for this chapter. Fine grade sequence of the appearance of SES-characters is indicated by numbers within each stage. For illustration, see Fig. 4.9. For character descriptions, see Werneburg (2009) and https://en.wikipedia.org/wiki/Standard_Event_System.

SES stage	SES characters	% in relation to opening of the eyelid
1.	(1) Primitive streak, (2) 1–5 somite pairs	11.4–14.7
2.	(1) 6–10 somite pairs, (2) head bulbus, (3) neural fold begin to close	14.8–15.7
3.	(1) Anterior cephalic projection, (2) otic pit, (3) bud of the 1st pharyngeal (mandibular) arch, (4) 11–15 somite pairs, (5) optic vesicle	17.2–18.5
4.	(1) 21–25 somite pairs, (2) 1st pharyngeal slit, (3) olfactory pit (placode), (4) 2nd pharyngeal (hyoid) arch, (5) 3rd pharyngeal slit, (6) otic vesicle, (7) coiling of the trunk, (8) 2nd pharyngeal slit, (9) ventricle bulbus, (10) ventricle is S-shaped, (11) forelimb ridge (wider than long), (12) maxillary process of the 1st pharyngeal (mandibular) arch reaches posterior level of the eye, (13) posterior neuropore closed, (14) anterior neuropore closed, (15) tail bud, (16) 3rd pharyngeal arch	20.3–23.4
5.	(1) 31–35 somite pairs, (2) 4th pharyngeal slit, (3) cervical flexure of 90°, (4) mandibular arch reaches posterior level of the eye, (5) hind limb ridge, (6) maxillary process reaches midline of the eye, (7) lens vesicle (placode)	23.4–25.0
6.	(1) Maxillary process reaches anterior level of the lens, (2) 4th pharyngeal arch, (3) nuchal fold, (4) 36–40 somite pairs, (5) forelimb bud (as wide as long), (6) forelimb apical epidermal ridge (AER), (7) external nares, (8) optic fissure, (9) mandibular arch reaches midline of the eye, (10) hind limb bud-shaped	25.0–27.5
7.	(1) Maxillary process at anterior level of the eye, (2) trunk coiling disappeared, (3) hind limb AER, (4) forelimb elongated (longer than wide), (5) 41–45 somite pairs, (6) mandibular arch reaches anterior level of the lens	27.8–28.6
8.	(1) 46–50 somite pairs, (2) forelimb paddle-shaped, (3) hind limb elongated, (4) mandibular arch reaches anterior level of the eye, (5) contour lens/iris	28.7–30.1
9.	(1) Hind limb paddle-shaped, (2) otic capsule inconspicuous, (3) pupil forms, (4) digital plate at forelimb, (5) somites become hard to count	30.2–32.2
10.	(1) Digital grooves on forelimb, (2) pharyngeal slits closed, (3) digital plate at hind limb, (4) maxillary process fuses with frontonasal process, (5) pinna fold, (6) mandibular arch reaches the level of the frontonasal process, (7) elbow in the forelimb, (8) nose openings are surrounded as nostrils	33.1–34.5
11.	(1) Digital serrations at the forelimb, (2) thoracal bulbus disappeared, (3) digital grooves on hind limb, (4) lower eyelid, (5) hair follicles on the head	34.5–36.3
12.	(1) Eyelid is at the level of the scleral papillae (between eye edge and lens), (2) digital serration at the hind limb, (3) eyelid has begun to overgrow the eye, (4) mammary anlage, (5) first finger (longer than wide), (6) knee in the hind limb, (7) cervical flexure disappeared	36.6–39.5
13.	(1) First toe, (2) eyelid reaches the ventral border of the lens, (3) mandibular arch reaches occlusion point with upper jaw, (4) tongue is protruding, (5) mesencephalic head projection disappeared	39.9–42.7
14.	*Fetal allometric growth increasing.* (1) eyelid covers more than half of the eye (eye closure), (2) first claw on forelimb, (3) first claw on hind limb, (4) vibrissal hairs on the snout	43.3–65.7
15.	(1) Hairs on the neck, (2) birth	85.4–86.4
16.	*First postnatal stage.* (1) hairs appear on forelimbs (proximal), (2) back, (3) top of the head, (4) hind limbs (proximal), (5) tail, (6) hind limbs (distal), (7) belly (dorsal part), (8) throat, (9) forelimbs (distal), (10) belly (ventral part).	87.4–97.8
17.	*Second postnatal stage.* (1) eyelids open	100

the placental mammal ancestor. We found, for example, that the neonates of the earliest placental mammals were altricial, almost naked and the eyes were still closed. In contrast to all marsupials, the limbs were evenly developed at birth (Fig. 4.10, SES-stage 15; see also Werneburg *et al.* 2016).

4.10 Gestation

At oviposition, monotreme egg size is less than two centimeters in diameter. Total gestation in the short-beaked echidna, *Tachyglossus aculeatus*, lasts for 17–27 days. At oviposition, the embryo has already developed an embryonic stage comparable with that of a chicken embryo at about 50 hours of incubation, taking a structured neural anlage, formed eye vesicles, 19–20 somite pairs, and a formed head shield of the proamnion as references (Semon 1894a–d, Hamburger and Hamilton 1951, Starck 1995, Werneburg and Sánchez-Villagra 2011). After oviposition, one or two eggs (Tab. 4.2) are incubated in the pouch(es) (Figs. 4.3 D and 4.13 B) for about 10 days; a similar amount of time can be assumed for the western long-beaked echidna, *Zaglossus bruijni* (Hayssen *et al.* 1993).

Intrauterine gestation usually lasts for 10–14 days in the platypus, *Ornithorhynchus anatinus*. After egg lay, normally two eggs are incubated in hollows for a further 7–14 days (Hayssen *et al.* 1993, Hughes 1993, Manger *et al.* 1998). Those hollows end in a chamber, which can have some grass inside (Fig. 13A). The platypus has an incubatorium, in which the egg is laid (Zeller 2004b). The fact that monotremes lay eggs was first recognized 93 years after their discovery by Europeans in 1884 (Caldwell 1884; see also Griffiths 1978). Australian Aborigines knew but were disbelieved by early European settlers (Moyal 2004). In contrast to sauropsids, a mineralized outer egg layer is assumed never to have been present in mammalian ancestors (Stewart 1997, Laurin *et al.* 2000), although monotreme oviparity is certainly inherited from amniote ancestors (Starck 1995). The ancestral amniote mode of reproduction is hard to characterize. Furthermore, it is not clear to what degree reproduction and development can be homologized between monotremes and sauropsids (Storch and Schröpfer 2004).

Incubation takes about 10 days in all monotreme species. The exact length of gestation of the platypus is unknown, partly because it is thought that the female can store sperm after copulation.

Marsupials have a very short gestation period, varying between 11 and 45 days (de Magalhães and Costa 2009, Clauss *et al.* 2014), with a mean of about 28 days (Tab. 4.2). Consequently, the marsupial neonates are born at a very early developmental stage, and a part of morphogenesis does not occur in utero but while the young is attached to the teat of the mother, often within a pouch (marsupium) (Tyndale-Biscoe 2005). In most cases, gestation is relatively shorter in marsupials when compared with placental mammals of similar size (Starck 1995).

In placental mammals, the gestation length varies strongly among species (Clauss *et al.* 2014). The shortest gestation occurs in the golden hamster, *Mesocricetus auratus*, and the common shrew, *Sorex araneus*, at about 2 weeks and the longest is documented for the African elephant, *Loxodonta africana*, at about 2 years (Tab. 4.3).

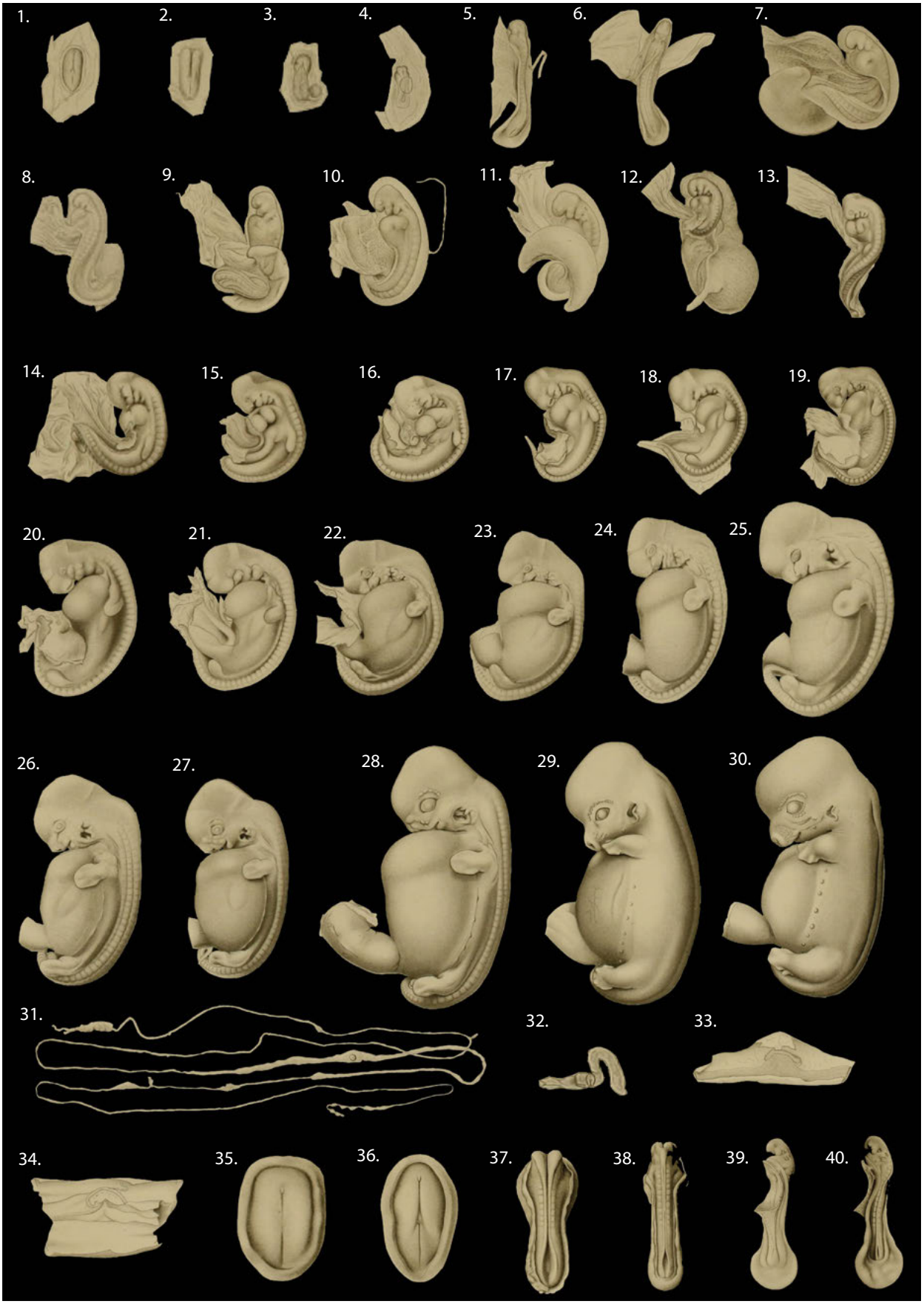
4.11 Delivery

Monotreme hatchlings have a size of 10–15 mm, are naked, and have closed eyes. The forelimbs are further developed than the hind limbs; however, monotreme hatchlings are not as altricial as marsupials are. Like marsupials, monotremes have large heads and forelimbs at birth, and urine is produced in their mesonephros (Zeller 2004b). They breathe via the primary branching of the bronchia, which is covered by respiratory epithelium before the alveoli and the bronchial branching are fully formed, which occurs postnatally (Starck 1995). Eyelids open about 12 weeks after hatching in the short-beaked echidna *T. aculeatus* (Vaughan *et al.* 2011) and after 11 weeks in the platypus *O. anatinus* (Manger *et al.* 1998).

Around hatching, monotremes have a true egg tooth and a keratinous egg tooth (caruncle) with an ossified part (os carunculae). The egg tooth is formed by the enamel organ and tooth papillae, and it is made up of dentine covered by a thin enamel layer. The caruncle develops as an independent new organ and later fuses with the processus ascendens of the premaxillary. Monotremes are the only vertebrates in which both a true egg tooth and caruncle are present. Squamates have just a true egg tooth; the other sauropsids have a horny egg tooth and their caruncle is only an epidermal structure (Starck 1995). Egg tooth and/or caruncle are used by the hatchling to tear up the eggshell from inside. In marsupials, rudiments of both structures can be found in embryos of some species.

Contrary to mammals, viviparity evolved several times independently in fishes and squamates, permitting inferences on the evolutionary adaptations and constraints involved using comparative methods (Blackburn 2015).

The sudden transition from fetal to postnatal life in therian mammals is correlated with crucial physiological changes in, for instance, the circulatory system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(Fig. 4.12 A–B) and basic metabolism. The neonate has to be prepared prenatally to perform all required functions at birth.

In the embryo, absorption of oxygen and nutrition is enabled through maternal blood circulation in the placenta (Fig. 4.12 A). Conversely, carbon dioxide and waste products are excreted through the placenta. The lungs are not yet unfolded in the fetus, and the blood circulation is not fully developed. Oxygenated blood in the fetus comes from the placenta through the vena umbilicalis, the ductus venosus, and the vena cava caudalis and enters the right atrium of the heart. Afterward, the blood passes through the foramen ovale, an opening in the septum of the right atrium. It floods into the left atrium, then into the left ventricle, and finally into the aorta. Because of the high resistance of the pulmonary vessels, only a minor fraction passes through the right ventricle and the lung circulatory system. Arteries originate from the aorta that direct toward the anterior part of the body, which facilitates a comprehensive blood support of the brain. On its way back from the head region, the right auricle receives deoxygenated blood from the vena cava cranialis. This blood does not enter the foramen ovale and mixes only slightly with the blood of the umbilical vein. Instead, it enters the aorta pulmonalis through the left ventricle. Throughout fetal life, the pulmonary artery has an open connection with the aorta through the ductus arteriosis Botalli. The lungs are still unfolded, and their capillaries provide a large resistance to flow. As a result, most of the blood reaching the first part of the aorta pulmonalis enters the aorta descendens through the ductus arteriosus. From there, it reaches the placenta through the umbilical arteries.

At birth, the pressure of the umbilical vein and the right atrium decreases due to the tearing of the umbilical cord and the fall in pulmonary resistance following expansion of the lungs. The pressure on the left side of the heart increases beyond that on the right side. This results in the closure of the foramen ovale by a preformed valve (Fig. 4.12 B). This valvula foraminis ovalis is pushed from the left side onto the thickened edge of the foramen. During the first breathing cycles of the neonate, triggered by the increased oxygen content of the blood, the ductus arteriosus is closed by the contraction of its smooth arterial musculature. The blood of the right ventricle now completely enters the capillary net of the lungs through the aorta pulmonalis. Here it becomes oxygenated and flows back to the left atrium through the pulmonary veins.

The therian neonate has to be able to breathe directly after birth. Therefore, the musculature of the thoracic wall and the diaphragm need to be fully developed at birth. The bronchi and the alveolar tree of the fetus are filled with liquid during gestation. This liquid is excreted by the alveolar epithelium. The amniotic fluid is separated from the lungs by the ventilator mechanism of the larynx. At birth, the cylindrical alveolar epithelium is flattened by the pressure of the incoming air. The remaining liquid is quickly resorbed.

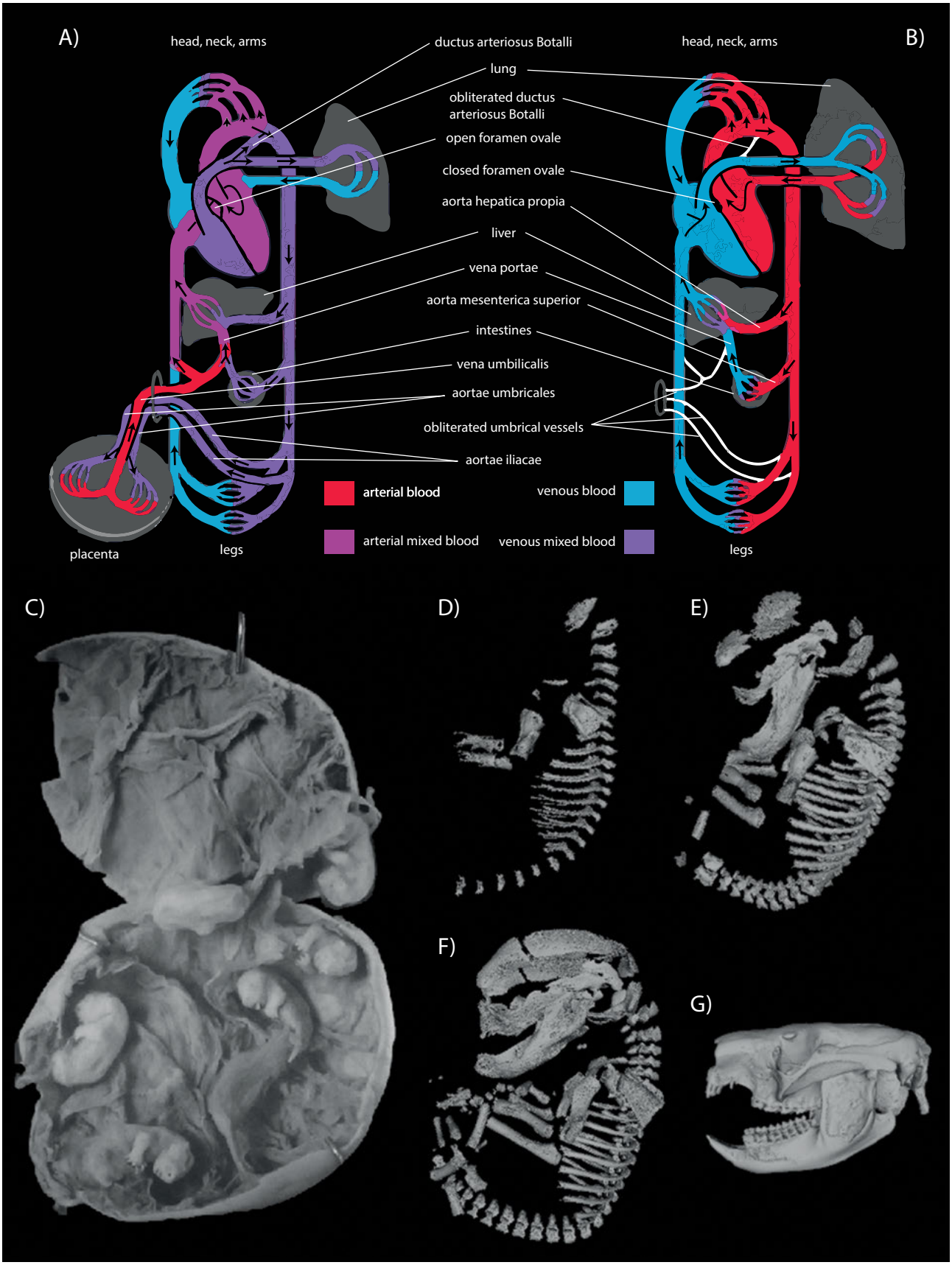
The urinal waste of the fetus passes the placenta and is excreted along with the maternal urine. During fetal life, kidney function is not required. At the end of gestation, excretion of urine into the amniotic fluid can occur. In therians with a large allantois, urine is excreted through the urachus, which is a specific part of the allantois, particularly in early phases. Later on, urine excretion may also occur through the urethra into the amniotic fluid.

Amniotic fluid is ingested by the fetus (500 ml/day in humans). The fluid quickly passes the esophagus, stomach, and small intestine, and most of the water is resorbed. Other contents, shed epithelial cells and bile pigments, are excreted as dark meconium at the first excretion after birth.

In marsupials, birth takes place through the unpaired birth diverticulum (not through the paired vaginae), which opens into the urogenital duct.

The closure of the eyes before birth or hatching in amniotes is an evolutionarily ancestral feature (Fig. 4.14 B–G). Compared with non-amniote vertebrates, mammals and sauropsids experience a drastic environmental change from an aquatic milieu in the egg or the uterus to atmospheric conditions after delivery. Therefore, the eyelids are usually closed during fetal development to protect the incompletely developed eye at birth (for exceptions, see Müller 1972–1973; Fig. 4.14 B–G). Tear glands still need to be developed, and the visual system has to be able to process visual information. The transitory closure of eyelids, similar to the closure of the ear and mouth openings, is enabled by cells of the intermedial layer of the epidermis (Fig. 4.14 B). The secondary opening later in development is facilitated by the cornification of those cells. The transitory closure of the nose is based on peridermal adherence (similar to the epitrichium, a peridermal layer covering the sense organs in marsupial pouch young).

◂ **Fig. 4.11: Historical drawings of the embryogenesis of the domestic pig, *Sus scrofa*.** Compiled from Keibel (1897). Numbers of embryos correspond to the original publication.


A)
head, neck, arms
ductus arteriosus Botalli
lung
obliterated ductus arteriosus Botalli
open foramen ovale
closed foramen ovale
aorta hepatica propia
liver
vena portae
aorta mesenterica superior
intestines
vena umbilicalis
aortae umbricales
obliterated umbrical vessels
aortae iliacae
placenta
legs
arterial blood
venous blood
arterial mixed blood
venous mixed blood
B)
head, neck, arms
legs
C)
D)
E)
F)
G)

4.12 Early marsupial postnatal life

Although all marsupials are altricial at birth, different degrees of altriciality can be distinguished (Fig. 4.14 E–H) (Hughes and Hall 1988, Smith 2015). In dasyurids and the honey possum *Tarsipes rostratus* (Diprotodontia), neonates have a neonatal weight of 3–20 mg (Tab. 4.2). Neither eye primordia, eyelids, nor retinal pigmentation are visible at birth. The tongue consists of multinucleate tubes and immature striations. The lungs show few partitions with superficial capillaries, and the metanephridic kidney is only represented as a ureteric bud. The metacarpals of the forelimbs are just differentiated and the primordial hind limb is an undifferentiated paddle (grade 1) (Fig. 4.14 E, Fig. 4.9–18 b).

In peramelids, didelphids, and brushtail possums *Trichosurus* (Diprotodontia), neonates have a neonatal weight of 100–300 mg (Tab. 4.2). Eye primordia and pigmentation are visible. The tongue muscles show striations, lung development has progressed, and the metanephric kidney is represented by a primitive ureter and a secondary branching (grade 2) (Fig. 4.13 F).

In kangaroo species (Macropodidae, *Macropus*), neonates have a birth weight of 300–900 mg (Tab. 4.2). Their eyes are prominently primordial, and a pigmented ring has developed. The tongue muscles show mature striations, and the lungs are large and highly subdivided and vascularized. The metanephric kidney has developed terminal branches and collecting ducts (grade 3).

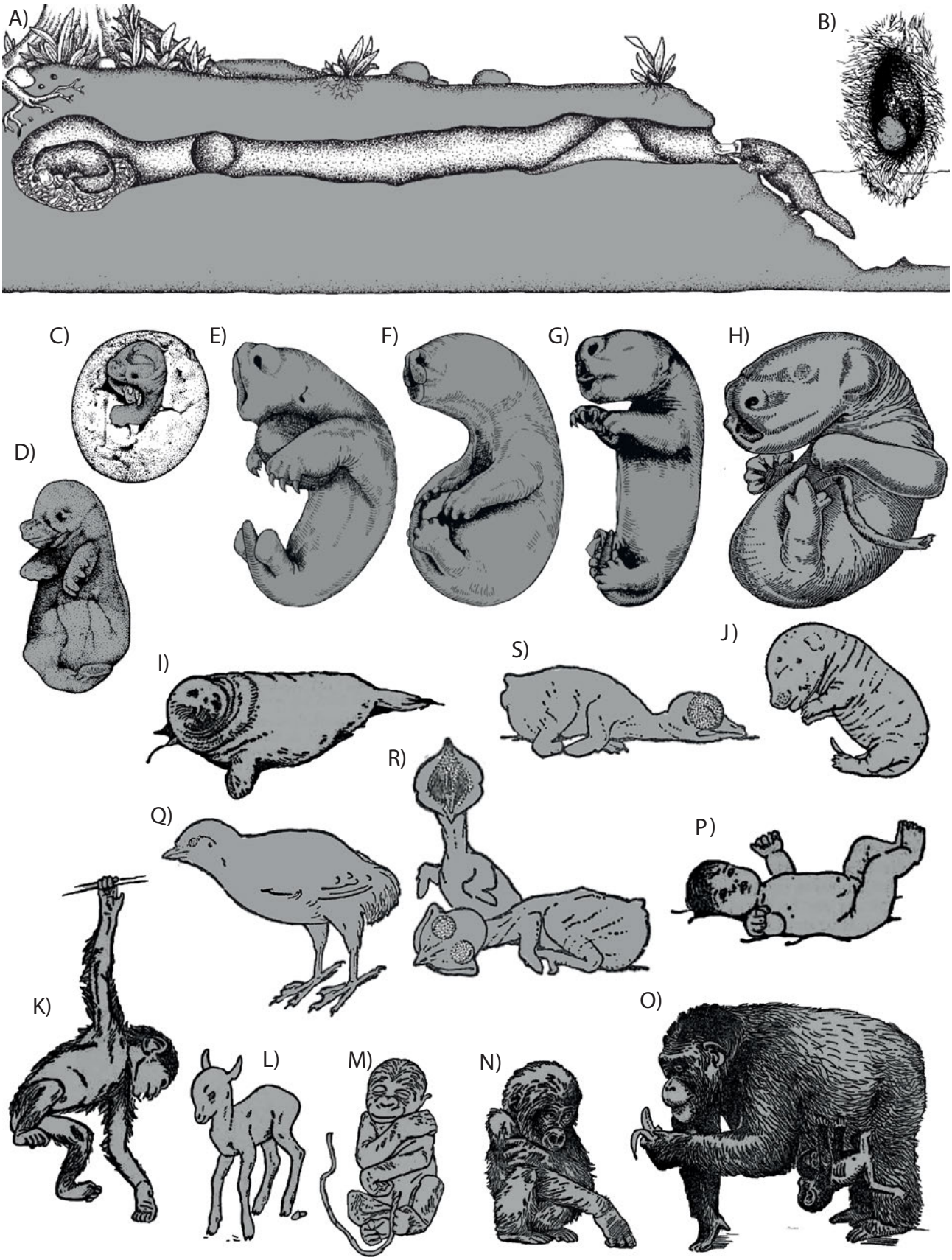
The different grades of altriciality that can be found among marsupials do not represent a phylogenetic gradation (Smith 2015). The intermediate grade (grade 2) appears to be ancestral and is the most widespread marsupial condition. The ultra-altricial condition (grade 1) evolved twice, whereas the most advanced condition is highly derived (grade 3).

It is important to note that neonate marsupials are not only characterized by immature development. As outlined below, several anatomical features (temporary closure of eyes and ears, deciduous claws on the forelimbs, and mesonephric kidneys) represent characters that are absent in the adult. Instead, these are functional adaptations to early postnatal life conditions, and they belong to the ancestral condition inferred for marsupial ontogeny (Maier 1999, Zeller 2004a).

Marsupial birth is characterized by the transfer of the neonate from the mother's urogenital sinus to the teat, which in most species is located inside a pouch (Hughes and Hall 1988, Gemmell *et al.* 2002). The mother has only little influence on the journey of the neonate (Zeller 2004a). In macropodids and the brushtail possums *Trichosurus*, the mother moves into a sitting position during labor. The tail turns ventrally, and the genital opening is directed toward the pouch (respectively toward the teats). In other marsupials, such as bandicoots and dasyurids, the mothers are also known to position the genital opening in such a way as to facilitate the neonate's transfer to the pouch. The neonate frees itself from the egg membranes with the help of the keratinous, deciduous claws of its forelimbs, which are replaced by permanent claws later in development. Some marsupials do not climb at birth (Ashwell and Shulruf 2014).

Altricial marsupial neonates are highly vulnerable after birth and, therefore, need to reach the teat as soon as possible to survive. This is accomplished through sinuous contractions of the para-axial musculature of the torso during which the head swings in a trial and error mode to find the teat. These movements are already performed in utero (Drews *et al.* 2013). The neonates exclusively maneuver themselves using their forelimbs, in which functioning chondrified elements are present and which are at a much further stage of development than the paddlelike hind limbs. Digitopalmar prehension and the above-mentioned deciduous claws in the forelimbs are further adaptations for this critical journey directly after birth. In certain marsupials (some didelphids), the requirement for the neonates to reach a teat as quickly as possible is further increased by the litter size being larger than the number of teats, meaning that neonates that are not able to attach to a teat before they are all occupied by siblings die soon thereafter (Hughes and Hall 1988, Tyndale-Biscoe 2005, Bininda-Emonds *et al.* 2007). In macropodids, the neonate crawls up the fur of the mother and reaches a teat within about five minutes (Starck 1995). The neonate most likely

◂ **Fig. 4.12: Perinatal anatomy.** Blood circulatory system in fetal (A) and postnatal mammals (B). Arrows indicate the direction of blood flow. Note the integration of the placenta into the circulatory system, the open foramen ovale, and the admixture of blood in the fetal condition (A). After birth, the foramen ovale is closed, the umbilical vessels and the ductus arteriosis Botalli are obliterated, and a clear distinction between arterial and venous blood becomes apparent (B). Modified after Deetjen and Speckmann (1999). (C) Opened uterus of the brown four-eyed opossum, *Metachirus nudicaudatus*, with six fetuses connected to their placentas. (D–G) Four developmental stages of postnatal skeletal development in the koala, *Phascolarctos cinereus*, visualized using microcomputed tomography; D–F = pouch young, G = adult skull. Note that in marsupials, the forelimbs ossify much earlier than the hind limb. Specimens are from the Embryological Collection Berlin (C: MS38, D: 487A, E: 485A, F: 484) and the Zoological Collection Berlin (ZMB: 36036).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finds its way to the pouch through olfaction, smelling compounds produced by apocrine sweat glands at the mother's teats. Only the teats to which young are attached will keep up milk production, and the other teats return to a resting mode (Fig. 4.14 Jd). The secreting glands grow up to six times their resting size during lactation, and the teats also grow more than double in size (Fig. 4.14 I, Ja, c).

Embryos of placental mammals develop within the regulated environment of the uterus, and vital functions such as breathing, blood circulation, digestion, and excretion are enabled by the placenta. In marsupials on the other hand, the very short gestation period, the high altriciality at birth, the journey of the neonate to the mother's teat, and the life in the pouch result in several specific adaptations in the neonate in order to manage these functions and survive outside the uterus. These specific adaptations that enable the extreme marsupial life history will receive particular attention in the following pages and should not be dismissed as simple results of an abbreviated intrauterine development and acceleration of some organ structures (Starck 1995, Weisbecker 2015, Weisbecker and Beck 2015).

In marsupials, eyes and ears are at an embryonic stage at birth (Fig. 4.13 E–H, Fig. 4.9–18 b). However, the olfactory system is well developed with a functional epithelium and innervation to detect the mother's teat. Taste buds have only been found in certain species (Hughes and Hall 1988). Eyelids and eye glands are not yet developed at birth. The jaws are relatively well developed and can open widely before reaching the teat. After that, the eyes, ears, and mouth angles are covered by a peridermal epitrichium for protection. This also enables a lateral closure of the mouth for effective suckling and prevents the pouch young from detaching from the teat, which is further prevented by a bulbous swelling of the teat inside the mouth, effectively anchoring the neonate to the teat (Fig. 4.14 H–I, Jb). The epitrichium starts disappearing in the third part of pouch life (Hill and Osman-Hill 1955, Starck 1995) (around Fig. 4.9–29).

The cortex cerebri of the brain is not yet fully developed, and the pyramid pathways are not yet differentiated. At birth, marsupials (studied in the mouse opossum, *Marmosa*, and the quoll, *Dasyurus*) lack cranial nerves II–IV and VI, cerebral commissures, eye pigments, and eyelids (Müller 1972–1973; Smith 1997).

In the lungs of marsupial neonates, vascularized sacs (pseudo-alveoli) are present at the perinatal stage, and only initial branching of the bronchi is present. The lungs can vary in development between a small respiratory cavity divided by simple partitions with superficial vascularization and much larger lungs, with many subdivisions and richly vascularized and cartilaginous trachea. In groups with large lungs, which correlate with neonatal mass, such as in the kangaroos (*Macropus*), the chest is barrel shaped (Hughes and Hall 1988). Coupled with large vascularization of the rump wall, breathing can also occur via the skin, which is very thin and moist. This has been well studied for some dasyurids (Mortola *et al.* 1999).

The heart ventricles are incompletely separated in marsupial neonates but are able to maintain their circulatory function. Temperature regulation starts relatively late during postnatal development and is first fully developed at weaning. Therefore, a close association to the mother is needed for the neonates to maintain their body temperature.

At birth, the metanephros of the kidney is not yet developed in marsupials. Urine excretion happens via the mesonephric (Wolffian) duct.

The pharyngeal area is well developed in marsupial neonates, including cartilaginous and musculatory structures and an epiglottis. The tongue and the mouth floor musculature are highly developed at birth. Together with the peridermal epitrichium, these are adaptations for attachment to the teat and controlled suckling. As the esophagus is not functionally developed, it is believed that swallowing at this stage is also performed by these structures. The stomach is the primary absorptive organ at birth. Although the small intestines seem functional, the large intestines are not as they are lined by an unspecialized epithelium (Hughes and Hall 1988). The nasal skeleton, larynx, and bronchial cartilages are very stable in the neonate and prevent the closure of the airway. As suckling

◂ **Fig. 4.13: Hatching and birth in different mammalian species.** Perinatal life of monotreme mammals: (A) Breeding burrow of the platypus, *Ornithorhynchus anatinus,* with the parents; (B–D) egg in the pouch (B), hatching process (C), and neonate (D) of the short-beaked echidna *Tachyglossus aculeatus*. Neonate marsupial mammals: (E) "ultra-altricial" marsupial *Dasyurus viverrinus* (eastern quoll) (see also Fig. 4.9; (F) "intermediate" marsupial *Monodelphis domestica* (grey short-tailed opossum); (G, H) "advanced altricial" marsupials *Macropus eugenii* (tammar wallaby; G) and *Macropus rufus* (red kangaroo; H). Altricial placental mammals: the neonate harbor seal *Phoca vitulina* (I) and the house mouse *Mus musculus* (J). Precocial placental mammals: a very young chimpanzee *Pan troglodytes* already climbing (K), a neonate domestic goat *Capra hircus* (L), a late fetus of *Gorilla gorilla* with well-developed limbs (M), gorilla neonate with adultlike limb proportions (N), gorilla young clinging to the mother's fur (O). Secondarily altricial placental mammal: the human, *Homo sapiens* (P). Precocial (Q) and altricial (R, S) birds. A, modified after Zeller (2004a, b); B–D, after Maier (1999), E–G, after Smith (2015), H, after Portmann (1959); I–J, L–Q, after Portmann (1944).

and breathing must work in parallel, the opening of the larynx extends above the muscular palate (velum) and reaches the nasopharyngeal space. In that way, the milk can flow laterally to the larynx into the esophagus (Zeller 2004a). The tongue is grooved to allow suckling through a so-called pump-sucking motion, which basically works like a piston. The secondary palate, diaphragm, and epiglottis are fully formed at birth (Lillegraven 1975, Maier 1999).

The short gestation period means that at birth marsupials have no ossification centers, and in most postcranial skeletal elements (Clark and Smith 1993), ossification only starts in the first postnatal days of development (Gemmell *et al.* 1988, Weisbecker *et al.* 2008, Spiekman and Werneburg 2017) (Fig. 4.12 D–G).

The forelimbs, the facial region, and the rest of the anterior part of the postcranium ossify earlier in marsupials compared with placentals. Conversely, the ossification of the hind limbs and posterior part of the postcranium occurs later in development. This is in part a form of heterochrony, in which the development of the entire hind limb is delayed (Weisbecker *et al.* 2008, Sears 2009). The molecular basis of such differences is currently being studied (Keyte and Smith 2010, Sears *et al.* 2012). A comparison between monotreme, placental, and marsupial postcranial skeletogenesis showed that monotremes are more similar in this respect to placentals than to marsupials, indicating that marsupial skeletogenesis (and its hypothesized constraints) does not form the ancestral mammalian or therian condition (Weisbecker 2010, Werneburg *et al.* 2016). Because it is known that muscular activity can influence bone formation (Rot-Nikcevic *et al.* 2006), it has also been hypothesized that the high muscular effort in the anterior part of the body during the climb to the teat has a role in the earlier ossification of this part of the postcranium (Weisbecker *et al.* 2008).

In the marsupial neonate, the shoulder and thoracic apparatus, including shoulder and neck musculature, are already well differentiated. The ventral (coracoid) plate of the shoulder girdle forms a rigid brace for the free forelimbs and it starts to be reduced some time after birth.

A certain similarity of young monotremes and marsupials concerns the anatomy of the limbs (cf. Figs. 4.7–4.8). The forelimbs are stronger than the hind limbs; however, the monotreme hind limbs are only a little (about 1 day) behind the forelimbs in their development, in contrast to the highly immature stage of marsupial hind limbs (Manger *et al.* 1998). It is worth noting that also in placental mammals, the hind limbs are usually somewhat delayed in their development when compared with the forelimbs, which appears to be a general pattern of amniote development and may be derived from the early tetrapod mode of limb development, in which the forelimb ancestrally develops faster than the hind limb (Harrison 1969, Werneburg *et al.* 2016).

The orientation of monotreme limbs is very similar to that of marsupials at birth and also permits a digitopalmar prehension. After reaching the teat, the forelimbs are not used for holding on in marsupials. Monotremes, in contrast, lack teats and slurp the milk from milk fields of the mother and use their strong forelimbs to hold themselves on to the mother's fur (Manger *et al.* 1998). Therefore, the similarities of limb anatomy and proportion in monotremes and marsupials might be caused by different developmental constraints.

It has been suggested that the climbing behavior in neonate marsupials represents the major constraint for marsupial anatomy (Weisbecker 2015). The forelimbs are highly adapted for this unique mode of locomotion, which therefore prevents a great radiation of forelimb anatomy in marsupials. Placental mammals, in contrast, have little early developmental constraints on the forelimbs and a much higher degree of shape variance has evolved, including fins, wings, and elongated limbs with hooves. Consistently, in marsupial species with little climbing activity as newborns (e.g., bandicoots), more variation in forelimb (Weisbecker *et al.* 2008, Weisbecker and Nilsson 2008) and shoulder (Sears 2004) anatomy is recorded. The hind limbs of marsupials do not experience a comparable constraint in early life and, as such, a much greater variation of hind limbs can be found, even compared with the highly diverse placentals (e.g., jumping legs in macropodids).

Initial cranial ossification in marsupials seems to be influenced by another main functional constraint of the neonate, namely the attachment to the mother's teat (Goswami *et al.* 2016, but see Spiekman and Werneburg 2017). The premaxilla, maxilla, dentary, palatine, and pterygoid are generally the first cranial bones to ossify and subsequently show a faster growth rate than any other cranial bones. The endochondral elements of the skull ossify later, starting on average posteriorly and ending anteriorly in the following order: exoccipital, basioccipital, supraoccipital, basisphenoid, and orbitosphenoid. Skeletal elements of the visceral arches on the other hand first ossify anteriorly and then in a caudal direction, with the alisphenoid ossifying first, followed by the malleus, incus, and stapes. Compared with placental mammals, the bones surrounding the oral cavity (premaxillary, maxilla, dentary, palatine, and jugal) are accelerated, and the bones surrounding the braincase are decelerated (Clark and Smith 1993, Sánchez-Villagra and

Sultan 2002, Koyabu *et al.* 2014, Spiekman and Werneburg 2017). Consequently, neurogenesis is also delayed compared with placentals, both in its onset and in its progress of development (Smith 1994). Around birth, the premaxillae of both sides temporarily fuse above the palatal elements. In a few cases, an ascending process of connective tissue forms, which can also ossify in the wooly opossum *Caluromys* (Zeller 2004a). This pattern represents a recapitulation of an os carunculae.

The relatively late ossification of the middle ear bones and the periotic (Sánchez-Villagra *et al.* 2008) are likely a result of a striking developmental pattern in marsupials. At birth, marsupials resemble the jaw articulation of adult sauropsids between the quadrate and articular. However, it does not serve as a functional joint (Sánchez-Villagra and Sultan 2002). After attachment to the mother's teat, this jaw joint is eventually replaced by the mammalian, secondary jaw joint between the squamosal and the dentary. The quadrate and articular are redirected to form the incus and malleus, respectively (Maier and Ruf 2016). The strong attachment to the teat and consequent immobility of the jaw joint is probably essential for this development to take place (Lillegraven 1975).

The function of the primary jaw joint in marsupial neonates is not fully understood. The genuine anatomical similarities between marsupial neonates and adult sauropsids are certainly rooted in the evolutionary mechanics of heterochrony, without dredging up the historical baggage of "recapitulation" (Robert J. Asher, personal communication). It does not have a synchondrotic or syndesmotic connection with any intra-articular space. A movement of the primary jaw joint would be inexpedient as the neonate has to attach tightly to the teat and the lips are partly fused. When weaning occurs, the secondary jaw joint between dentary and squamosal is already well developed.

There are numerous anatomical autapomorphies of Marsupialia (e.g., fenestration of the palate, which therefore incompletely separates the mouth and nasal cavities; a large jugal bone that extends posteriorly all the way to the glenoid fossa of the skull), some of which may relate to the developmental adaptations and/or constraints of these animals (e.g., Goswami *et al.* 2016). Noteworthy is the median inflection of the angular process of the dentary bone, unique to marsupials, which has been discussed in the context of potential hearing function around the time of birth (Sánchez-Villagra and Smith 1997).

To buffer the forces experienced during suckling in marsupial and placental mammals, a nearly complete closure of the secondary palate at birth is developed. An early and strong chondrification of the nasal capsule and the formation of a relatively complete cartilaginous cranial side wall (commisura orbitoparietalis) can also be seen (Hüppi 2018). The latter supports the developing processus ascendens of the ala temporalis (secondary cranial side wall and precursor of the alisphenoid bone), which serves as an attachment site for jaw musculature and to stabilize the skull (Maier 1987). In that way, the large trigeminus ganglion is also protected (cavum epipterygum).

Cranial muscle development occurs quickly compared with cranial bone development in marsupials. Development starts shortly before birth, but most myogenesis occurs postnatally as the muscles start to be functional in the neonate. The onset of myogenesis precedes the onset of both skeletal development and the development of the central nervous system. On the other hand, the development of the peripheral nervous system and certain sensory nerves are also, like the craniofacial musculature, well developed shortly after birth (Smith 1994). The actual innervation of the face by terminal branches of the trigeminal nerve varies among marsupials, and therefore the amount of tactile sense in the face and its role in finding the pouch remains to a large degree unknown (Hughes and Hall 1988). Despite the short period of myogenesis, cranial muscle development can be divided in three groups. Before birth, the tongue, the mylohyoideus and pharyngeal muscles, and some muscles of the neck and shoulder develop first. The muscles of the first pharyngeal arch and most other craniofacial muscles follow about 12 hours later (in the case of the short-tailed opossum *Monodelphis*). The facial and ocular muscles form about 24 hours after this. Taken together, it seems that there is little heterochrony in craniofacial myogenesis among marsupials, and there are also few differences with the development seen in placental mammals (Smith 1994). In the neonate jaw, muscles develop, stabilize the region of the developing jaw joint, and support the tongue for suction pumping.

As a result of its developmental constraint, the facial diversity of marsupials is relatively low, as major snout-related bones are already differentiated to enable teat fixation and suckling at early stages, although prominent exceptions exist (Goswami *et al.* 2011). A faster growth of the snout (Sánchez-Villagra *et al.* 2008) and a delayed brain development (Smith 2001) appear to have no impact on adult brain size, which is very similar in marsupials and placentals, although unique exceptions exist among placentals (primates, cetaceans) (Weisbecker 2015).

4.13 Early life history of placental mammals

Like monotremes and marsupials, many placental mammals are primarily altricial. However, compared with the former, placental mammals have a more or less fully developed body at birth and the forelimbs and hind limbs are more evenly developed (Werneburg *et al.* 2016). In contrast to monotremes and marsupials, a greater variety of conditions are known at birth for placental mammals (e.g., Starck 1995, Gilbert 2006, Westheide and Rieger 2010), which might be correlated to the great diversity and ecological adaptations of that group. In extremely altricial placental species, the neonates have no fur. No species-specific coordination of locomotion has developed, closed eyes are present until several days after birth, incomplete thermoregulation as the fur is not fully developed, and fast postnatal growth takes place. In addition to altricial forms, precocial conditions exist, in which the neonate has operable eyelids, fully developed fur, and locomotion, and sense organs are already functional (Portmann 1944, 1959). There is no clear distinction between altricial and precocial species, as a broad continuum exists (Martin and MacLarnon 1985, Sánchez-Villagra and Sultan 2002, Werneburg *et al.* 2016).

Among placental mammals, an altricial condition at birth can be found in tupaiids, terrestrial carnivorans, lipothyphlans, most rodents, and lagomorphs (Fig. 4.13 I–J). The young are kept inside burrows and are periodically fed. Some precocial animals have no nest, but in the first days, the neonates do not follow their mother and rest in relatively open places (the hare *Lepus*, macroscelids, some rodents and pigs). This condition appears to represent a transitional form to full precocial behavior. True precocial young experience a long gestation period and follow their mother directly after birth. This behavior can be found in the fully aquatic taxa: whales (Cetacea; Fig. 4.3 G) and manatees (Sirenia), whose life in water requires complete adaptation to the aquatic milieu directly after birth. Large herbivores (Bovidae, Proboscidea, and Perissodactyla; Fig. 4.13 L), all hystricognath rodents (e.g., guinea pigs, *Cavia*), and some other rodents (spiny mouse, *Acomys*) exhibit precocity. In even- and odd-toed mammals (Cetartiodactyla and Perissodactyla, respectively), the necessity for long-distance travel in savannas favored neonates that can keep up with the step of the adults directly after birth. Hystricognaths are among the most precocial rodent species and in general rank among the most precocial placental mammals (Sekulić *et al.* 2006).

Clinging young are known for some arboreal and aerial placentals without a nesting site (Dermoptera, Chiroptera, and Primates). The young are always carried by their mother (Fig. 4.13 O). The same is true for the terrestrial pangolins and the giant anteater (*Myrmecophaga*). Clinging is a special case with both altricial aspects, namely relative dependency on the mother, and precocial aspects such as refined limb adaptation to hold on to the mother's fur.

Bats (Chiroptera) are intermediate between altricial and precocial conditions at birth (Kurta and Kunz 1987, Rasweiler and Badwaik 2000, Mess and Carter 2008, Koyabu and Son 2014). The young are carried by their mother in the first phase of life. Afterward, they are deposited in nursery roosts. The young usually attach to the teats or hold on to the fur of the mother. To clasp to the fur of the adult, hind limb ossification is accelerated in bat development (Koyabu and Son 2014). In some taxa, specialized teats are present in the inguinal region specifically developed for the young to cling on to. They do not secrete milk and the young have particularly formed milk teeth that enables them to attach to these specialized teats. Some male bats lactate (Francis *et al.* 1994).

Human children are born as secondarily altricial (Fig. 4.13 P) (Portmann 1944, 1959). Other primates give birth to relatively mature young, with fully developed fur, open eyes, and refined motor function (Fig. 4.13 K, M–O) and are more precocial than altricial in their birth condition. That humans derived from precocial ancestors is recognizable when comparing their neonate anatomy to typical, primarily altricial mammals. Most organs in humans are fully developed, including head hair, and the eyes are open at birth. Moreover, infants show the grasp reflex, a precocial feature, which is inherited from primate ancestors, as outgroup comparison in a cladistics sense prove (Fig. 4.13 P).

Truly precocial mammals, such as the cow, close their eyelids very early during embryogenesis and open their eyelids within the extended period of gestation some time before birth (Fig. 4.14 F). Humans close their eyelids at the end of the first trimester (Fig. 4.14 G). Within the fifth month, four months before birth, the eyelids open again. This is different to other primates. As such, the human has to be declared secondarily altricial at birth. It has been argued by Portmann (1944, 1959) that the first year of human development has to be understood as the extrauterine phase of fetal development, in which species-specific modes of locomotion and communication develop. This is related to the progressive process of brain folding and the establishment of an elongated learning phase in the infant after birth.

There seems to be a tradeoff between the size of the pelvic opening of the mother and the head size of the fetus,

which resulted in a physiologically preterm delivery. It has been argued that the pelvic opening could have coevolved with the fetus head size (Van Schaik 2016, Trevathan 2015). To some degree, this seems to be the case as documented by the fossil record (Zollikofer and Ponce de León 2010, Fischer and Mitteroecker 2015). Nonetheless, the effective bipedal locomotion of females was maintained (Gruss and Schmitt 2015), for which a particular pelvic anatomy was required and the pelvic opening therefore could not have widened extensively.

When compared to chimpanzees (*Pan*) as our closest living relatives, different accelerations (neotenic features) and extensions (hypermophoses) of postnatal ontogenetic traits can be observed in the human fossil record. Whereas one of the earliest hominin representatives, *Sahelanthropus tchadensis*, had an adult skull shape comparable to *Pan*, a progressing reduction of the facial skeleton evolved on the stem line to *Homo sapiens* (Fig. 4.15: boxes, Zollikofer and Ponce de León 2010). An absolute reduction is likely related to the progressing use of fire to process food, which then became easier to chew and less jaw muscle insertion area on the skull was needed. A relative reduction of the facial skeleton is also related to increased brain size for better cognition (tool processing, social interaction, language, metaphysical cognition) with *Pan troglydytes* having 365 cm³ and *Homo sapiens* 1350 cm³ brain volume (Fig. 4.15: boxes). Whereas skull shape of hominin newborns are relatively similar to *Pan*, the facial skeleton received its final shape relatively faster and brain growth was also accelerated to reach adult size (Zollikofer and Ponce de León 2010). Compared to *Pan*, the human brain, however, develops much longer and also body growth is extended. As a result, humans reach sexual maturity relatively late, which results in less evolutionary fitness. To circumvent this issue, humans wean their young relatively early compared to other great apes and intervals between pregnancies are shorter. This however, results in many non-independent young at the same time. To resolve this fitness disadvantage, hominins evolved a higher degree of allopatric behavior and particularly the grandmothers help raising the premature young (extended family). Largely increased social interactions and communications are among the key innovations for human evolutionary success (Van Schaik 2016). Life history traits are nicely mirrored in the sequence of dental eruption with the appearance of the first molar indicating the principal end of brain growth (followed by brain maturation), the appearance of the second molar indicating the end of the major somatic growth, and the appearance of the third molar indicating the begin of sexual reproduction (Fig. 4.15; Zollikofer and Ponce de León 2010).

4.14 Life of the infant

As mentioned before, milk glands are present in all mammals (even in some males as noted above). Milk glands are lobular, alveolar, apocrine glands, which are derived from sebaceous follicles. In monotremes, they are permanently associated with the hair follicles and they do not form teats but lactation fields instead, in which the excreted milk is collected (Fig. 4.3 D). The gland ducts develop into wide milk ducts in which milk is accumulated between lactation phases (Starck 1995).

The milk of monotremes has similar contents as the milk of placental mammals. However, like marsupials, there is a high concentration of iron in the milk of monotremes, which is much lower in placentals. Compared with placental mammals, both groups have small hatchlings or neonates with a correspondingly small liver. There is no change in the composition of milk constituents during the lactation period in monotremes (Jackson 2003).

The young of echidnas leave the furry pouch of the mother at an age of two months at the earliest. After that they are regularly fed for four to six days (Zeller 2004b). The platypus does not have a pouch (Hayssen *et al.* 1993). Its postnatal development is well documented (Manger *et al.* 1988, Fig. 4.8).

Lactation occurs for up to 200 days in the echidna and 105 days in the platypus (Zeller 2004b). However, weaning is a gradual process, which already starts a few months after hatching. During several weeks of lactation, the caruncle persists in the platypus, and it has been proposed that a rubbing of the caruncle on the mother's belly stimulates milk production (Griffiths 1978, Manger *et al.* 1998).

In therian mammals, the milk glands are separated from the hair follicles, although a connection is still visible in the ontogeny of some species. The number of teats generally corresponds to litter size.

After birth, the young searches for the teat and attaches to it via trial-and-error. The hairline of the mother helps during searching. Suckling on the teats (papillae mammae) stimulates the secretion of milk in the milk glands. Suckling is an active process of the young, which is enabled by a suction pumping mechanism (Maier 1999, German *et al.* 2000, Zeller 2004a, Krockenberger 2006).

As shown by fossil metatherians, the ancestral therian mode of tooth replacement after lactation was similar to that of extant placental mammals. As such, the unique mode of tooth replacement in extant marsupials is highly derived, and it has been correlated to the unique fixation to the mother's teat (Cifelli *et al.* 1996, Martin 1997; but see van Nievelt and Smith 2005).

The pouch of marsupials (Fig. 4.14 K) develops from the paired anlagen of the cranial ends of the genital buds (separated from the scrotum anlagen) and forms a permanent invagination of the skin through the dermal muscular tunic (panniculus carnosus) (Fig. 4.2 B). It is not homologous to the incubatorium (often also referred to as a pouch or marsupium) of female echidnas (Monotremata), as the latter is only a temporary formation, which develops from an unpaired folding of the abdominal wall. In marsupials, the pouch opening can be oriented in a ventral (Didelphidae and Dasyuridae), caudal (toward the cloaca; Peramelidae, Dasyuridae, and Phascolarctidae), or cranial (Macropodidae and Phalangeridae) direction. It is surrounded by the musculus sphincter marsupii, which fully closes during diving in the only semiaquatic marsupial (the water opossum, *Chironectes minimus*). Several small marsupials have no pouch or only a small crest around the teat field. In those species, the young are protected by the mother, and they are also tightly attached to the teats. It is not clear if a pouch belonged to the common ancestor of crown marsupials. It appears that it may have evolved several times independently (Zeller 2004a).

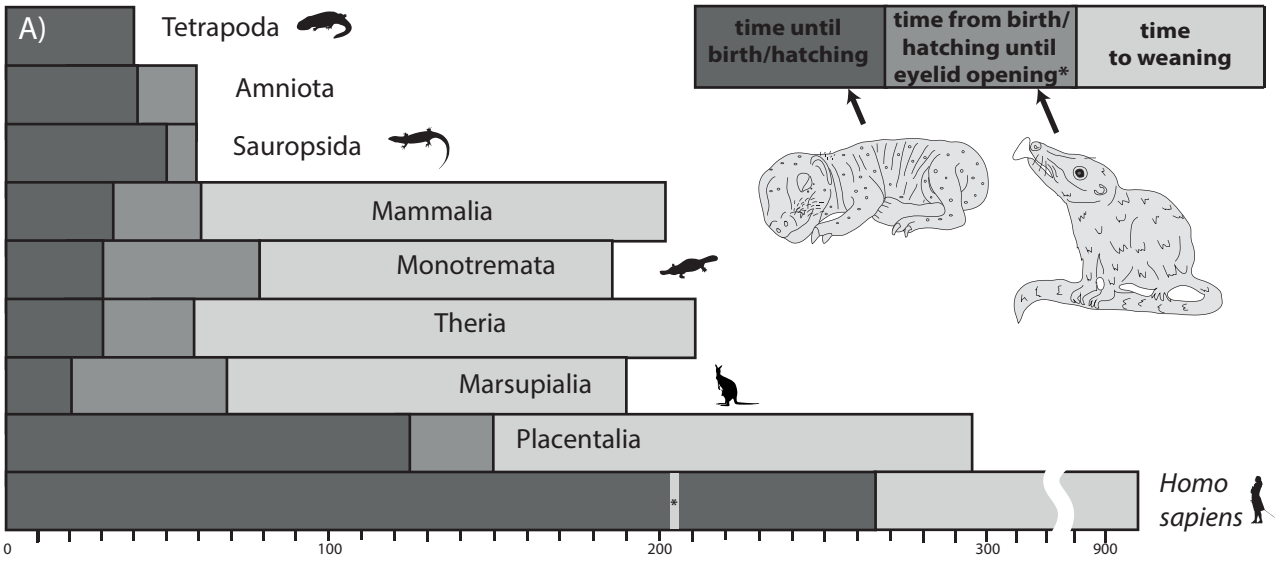
Males and females of all marsupial species have epipubic bones (ossa epipubica). These bones are also present in monotremes. As integrated elements of the abdominal wall, they play a role in locomotion and respiration (Reilly *et al.* 2009) and are fully independent of pouch formation. Epipubic bones and pouches are absent in placentals, but the former have been reported among stem eutherians (Novacek *et al.* 1997) and among mammaliaforms generally (Kielan-Jaworowska *et al.* 2004).

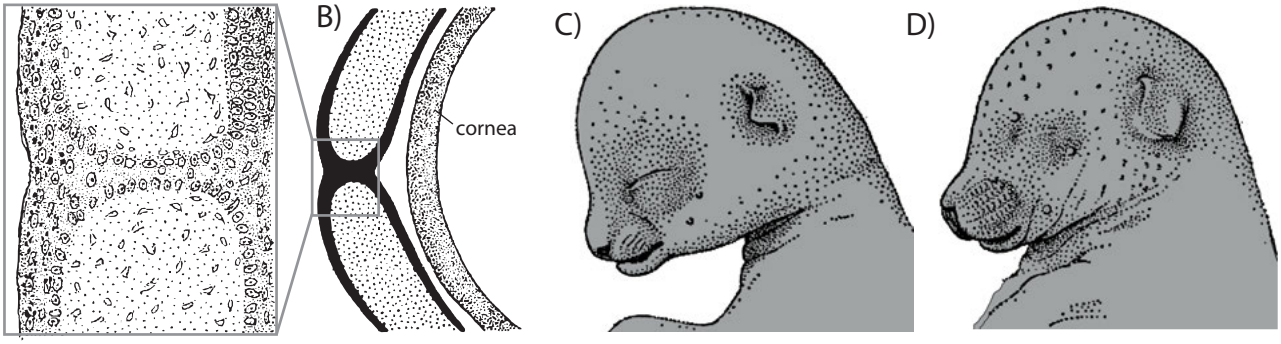
The lactation period of marsupials is an extreme multiplication of the gestation length (Fig. 4.14 A, Tab. 4.2). In marsupials, the milk glands are always situated on the ventral side of the body and consist of balled-up monoptychic gland ducts inside connective tissue with striated musculature (musculus ilio-marsupialis). One or more gland ducts exit into each teat. Plesiomorphically, a large number of teats is present (e.g., 25 in Didelphidae), but the count can be as few as two (southern marsupial mole, *Notoryctes typhlops*) (Zeller 2004a). In contrast to placentals, the teats in marsupials do not develop along a teat crest, but by a thickening of the epidermis, which contains up to six hair follicles (quoll, *Dasyurus*) with sebaceous gland and milk gland anlagen. In contrast to monotremes, the milk hairs fall out when sexual maturity is reached. In some marsupial species teat anlagen can also be found in males.

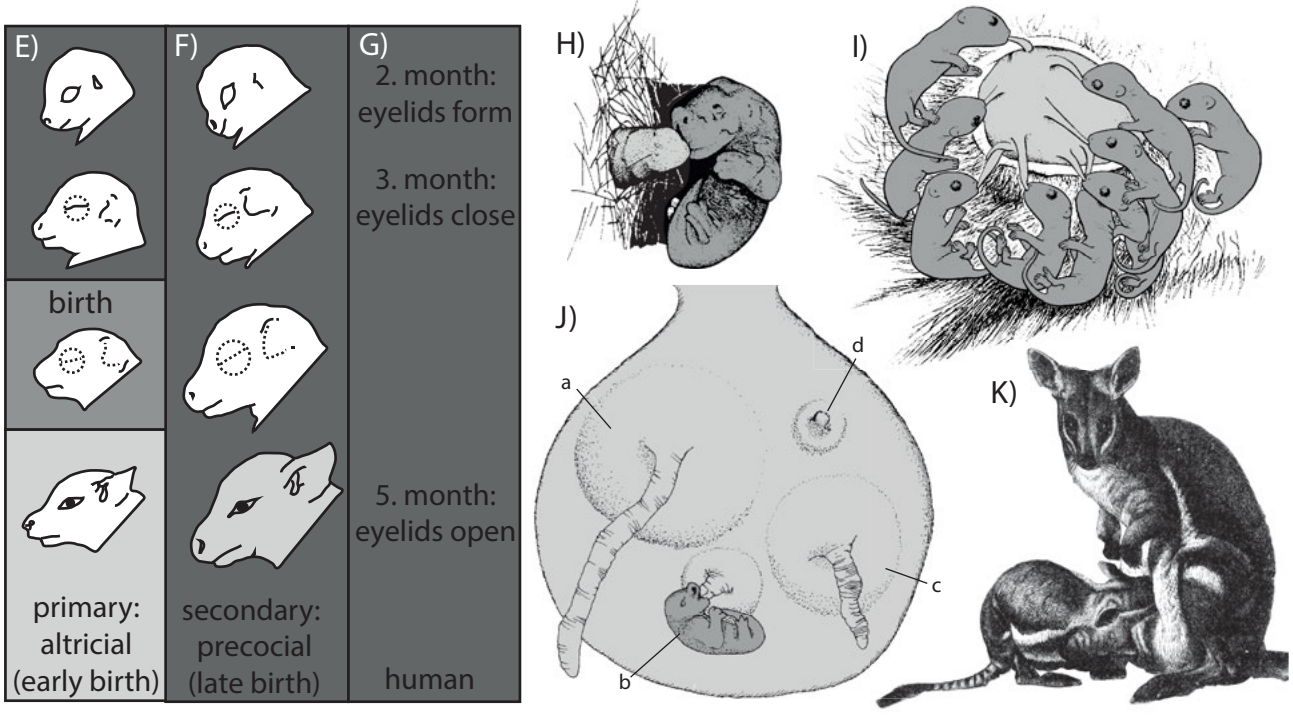
Marsupial lactation provides less nutritional energy per time unit (Krockenberger 2006) and correspondingly reduces maternal energy investment, which may be suited for harsh environments (Tyndale-Biscoe 2001). Much of organogenesis occurs while the young are attached to teats. At every growth stage, a different composition of the milk is necessary. Three phases of milk production are distinguished (Tyndale-Biscoe 2005, Krockenberger 2006). The older young (Fig. 4.14 K) get more protein- and fat-rich milk than the younger ones (Fig. 4.14 H–I and Jb). Marsupials can nourish several young of different ages in their pouch, each of which is associated to a different teat and receives specific milk contents (Fig. 4.14 J).

Lactation behavior is different among placental mammals. For example, the domestic pig *Sus scrofa* tends to lie down during lactation. Many precocial placental mammals, on the other hand, stand during lactation, whereas primate mothers sit and hold their young near the body (Storch and Schröpfer 2004). The contents of the milk vary among placental mammals; however, in contrast

▸ **Fig. 4.14 Postnatal life.** (A) Absolute time of the preweaning developmental periods as reconstructed for the major tetrapod and mammalian clades by Werneburg *et al.* (2016); legend in the right upper corner. The human is shown as a secondarily altricial species among placental mammals (* = timing of eye opening in the human occurs before birth). (B) Embryonic eye closure in altricial mammals with microscopic close-up of the lid adhesion. (C, D) Late embryonic heads in altricial mammals (C, kitten; D, shrew). The eyelids are grown together. The tip of the ear pinna, which points backward later on, is folded forward and fully fuses with the skin of the head. In panel D, this process is more advanced than in panel C. (E–G) Comparison of head development in mammals with different life history strategies. Primarily altricial mammals show closure of the eye and ear at birth (E). In precocial mammals, the eyelids open shortly before or around birth (F). Humans (G) have an extended prenatal development (see A*), but eye opening ancestrally occurs at an earlier point of development. Although humans have opened eyes at birth, they are not able to perform species-specific locomotion and communication and are altricial for the first year of postnatal life see Fig. 4.13P. Because humans evolved from precocial primates, they are called secondarily altricial. (H) Neonate of the red kangaroo *Macropus rufus* fixed to the teat. (I) Young of the opposum (*Didelphis*) suckling on teats. (J) Inside the pouch of an adult red kangaroo (*Macropus rufus*). (a) milk gland used by a young that already left the pouch, (b) milk gland with a neonate, (c) regression of the milk gland after weaning of a young at over 400 days, (d) reduction of an unused teat after progesterone stimulation during gestation. (K) Suckling young of the yellow-footed rock wallaby *Petrogale xanthopus* after leaving the pouch. B–D, after Portmann (1959); H, after Maier (1999); I, after a drawing of W. Maier; J, after Tyndale-Biscoe and Renfree (1987); K, after Brehm (1927).


A)
Tetrapoda
Amniota
Sauropsida
Mammalia
Monotremata
Theria
Marsupialia
Placentalia
Homo sapiens
0
100
200
300
900
time until birth/hatching
time from birth/ hatching until eyelid opening*
time to weaning


B)
cornea
C)
D)


E)
F)
G)
2. month: eyelids form
3. month: eyelids close
birth
5. month: eyelids open
primary: altricial (early birth)
secondary: precocial (late birth)
human
H)
I)
J)
a
b
c
d
K)

to marsupials, the milk of a species does not change much in its composition during lactation (Zeller 2004a).

In particular, altricial placentals (many murids, terrestrial carnivores, and strepsirrhines) show a complex retrieval behavior in order to change nesting sites in case of danger or parasite infestation of the nest. The mother or both parents carry their young by their head, fur of the nape, or ventrally at the flanks. In order to enable an effective weight distribution, the young fall into a passive carrying posture (at least in particular phases of development) (Storch and Schröpfer 2004). Whether a comparable retrieval behavior is present in marsupials is not clear (Hunsaker 1977), but it is most likely absent as it is in monotremes. In many arboreal and some terrestrial species, the young attach to the teats or to the fur of the mother during transport.

As in monotremes and marsupials, the period during which lactation ceases (weaning) is a continuous process, which can last for several weeks or months in placental mammals. Even after weaning, many placentals are not completely independent and may be supported by food supply, for example, in the form of shared resources by the parents (e.g., carnivorans and humans).

It is generally considered that teats first evolved in therian mammals and that the monotreme mode of lactation, slurping milk from the milk fields, represents a plesiomorphic condition. This is supported by the fact that no rudimentary trace of a teat has ever been found in monotreme development. However, it is worth noting that the tube-shaped mouth of echidnas and the horny beak of the platypus, already present upon hatching, is not convenient for suckling on teats and that slurping could therefore represent a derived feature in monotremes.

Monotreme hatchlings have an average relative mass of 0.08% compared with their mother. Marsupial neonates have 0.12% of the adult body mass, whereas placental mammal neonates already make up about 15% of the maternal mass. At weaning, the total average mass of monotreme litters relative to the mother is 50%, whereas it is 55% in marsupials and 59% in placental mammals. This shows that maternal investment of monotremes and marsupials is greater during lactation, whereas placental mammals invest most energy in placentation. However, the total investment is very similar between all three clades (Hayssen *et al.* 1985, Renfree *et al.* 2009).

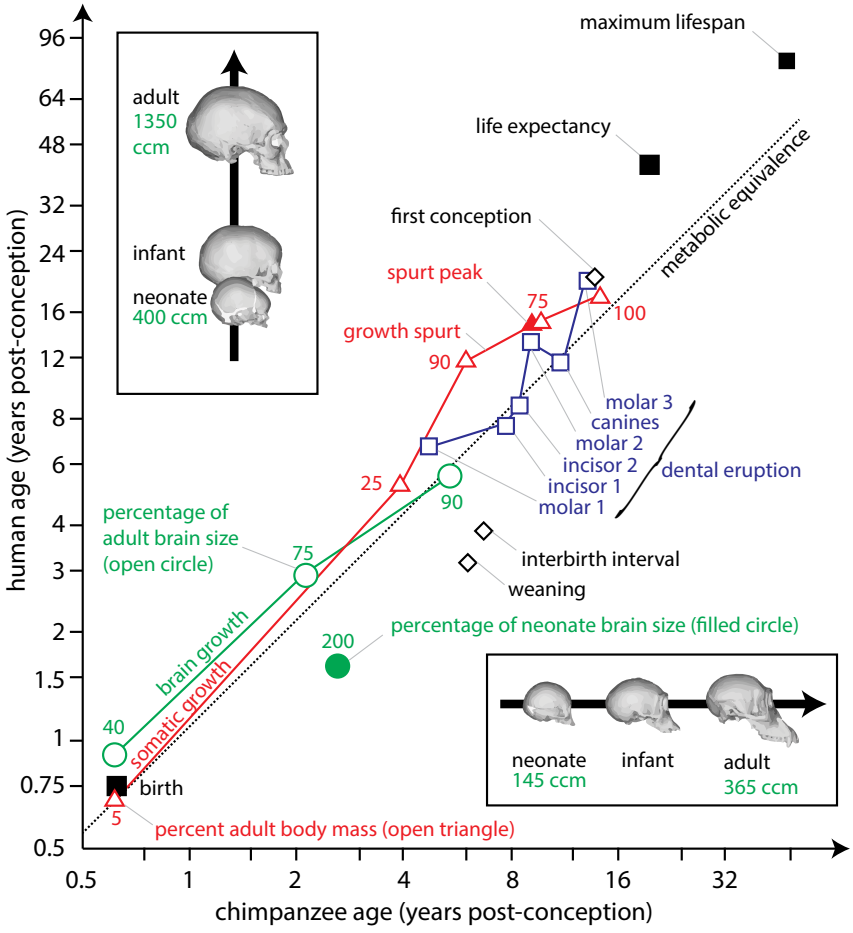


Fig. 4.15. Comparison of postnatal life history traits in the chimpanzee and human (modified from Zollikofer and Ponce de León 2010). Sequence of dental eruption in apes is: molar 1-incisor 1-incisor 2- molar 2 – (premolar 3, premolar 4)-canines-molar 3. In the human, it is: molar 1 – incisor 1 – incisor 2 – (premolar 3, canines, premolar 4) – molar 2 – molar 3. The sequence is dependent on shape of the face and food. ccm = cubic centimeter.

4.15 Life history evolution

Altriciality appears to be ancestral for all mammals because monotremes, marsupials, and most placental mammals have immature hatchlings or neonates (Portmann 1944, 1959, Starck 1995, Werneburg *et al.* 2016). Delivery at a premature stage and nourishing of the young with maternal histotrophs/placental nutrition and milk is tightly correlated with the inceptive reduction (Monotremata) and the elimination (Theria) of yolk in the eggs. Originally, the altricial lifestyle of mammals was apparently linked to a moderate litter size and breeding in a nest or burrow. Two modes of reproduction evolved, resulting from two types of selection, namely *k*-selection and *r*-selection (Pianka 1970), representing ends of a continuum. *R*-selection favors a large litter size and a trend toward fewer pregnancies in a life cycle (with an extreme of only one pregnancy in life, semelparity, as found within the broad-footed marsupial mouse *Antechinus*, for example). *K*-selection, on the other hand, favors a small litter size, which is compensated by intensive parental care and a long childhood, resulting in a relatively high proportion of young reaching sexual maturity. Litter size is correlated with individual longevity, body size, ecological factors, climate, food availability, and social behavior (Starck 1995).

The precocial lifestyle of several placental mammals has to be considered as derived given the well-nested position of these animals in the mammalian tree of life (Mess and Carter 2006, Meredith *et al.* 2011, O'Leary *et al.* 2013).

As mentioned above, a measure for the starting point of independency is the opening of the eyelids. In all precocial sauropsids, the eyelids open at hatching. In contrast, eyelids open long after birth or hatching in most mammals and in some birds, which is associated to their altricial life. Given that information, Werneburg *et al.* (2016) reconstructed the timing of eye opening in the last common ancestor of all amniotes in their phylogenetic reconstruction. They hypothesized that eye opening occurred several weeks after hatching in the amniote ancestor. A late eye opening does not necessarily mean that the young is underdeveloped at hatching (Sánchez-Villagra and Werneburg 2016). On the contrary, Werneburg *et al.* (2016) reconstructed well-developed limb, body, and head proportions. The closed eyes at hatching simply mean that the tear glands have not been developed enough to protect the eyes from drying out, and/or it means that the brain was not yet developed enough to process visual information.

Blind hatchlings require parental care and external food supply. In fact, there is some indication in the fossil record that parental care could have been present in early synapsids (Botha-Brink and Modesto 2007, 2009) and reptilian taxa (Piñeiro *et al.* 2012). Under this hypothesized reconstruction of the amniote ancestor, altriciality and parental care are plesiomorphic features for mammals.

It is most likely that polylecithal eggs, as in sauropsids and monotremes, were present in the amniote ancestor. Furthermore, the coincidental presence of polylecithal eggs and an altricial lifestyle in early amniotes is also not unlikely given that altricial (like precocial) birds also have polylecithal eggs.

Because of the morphological similarities between marsupial neonates and monotreme hatchlings, it has been suggested that oviparity was still present early in marsupial evolution and that the incubation of the eggs was later incorporated into the intrauterine period (Griffiths 1978, Starck 1995).

Viviparity never evolved in birds, probably because their highly vascularized lungs need some time to unfold before delivery (Duncker 1978). For that reason, they breathe in the air cell of the eggs (Fig. 4.5 G) shortly before hatching to permit a smooth "pulmonary transition" to the atmosphere outside the egg. Furthermore, the fact that birds and other non-marine archosaurs (and turtles, which may be archosaurs or at least very close to them) did not develop viviparity is likely related to their calcium metabolism (Gilbert 2006, Liu *et al.* 2017). Mammals, in contrast, do not have such limitations. Oviparity involves a large energetic investment of the mother at the moment when the eggs are produced. In viviparous forms, on the other hand, energetic investment is evenly distributed over a longer term (although huge variation exists, e.g., between marsupials and placental mammals). Monotremes represent an intermediate form in this regard, as they nourish their embryos with placental secretes (histotrophs) and the eggs are retained in the uterus for a longer embryonic time when compared with sauropsids. Viviparity is also beneficial to the endothermic physiology of therian mammals and protects the embryos from the danger of temperature fluctuations. In ectothermic reptiles, the retention of oviparity might have been the only possibility to survive in most cases because the body temperature of the mother fluctuates too much.

The strikingly different reproduction strategies of marsupial and placental mammals have been the source of debate on the origin of both groups and on their consequences for diversity and disparity patterns in evolution (e.g., Krockenberger 2006, Bennett and Goswami 2013, Sánchez-Villagra 2013). Placental mammals produce relatively mature young after a long gestation, whereas marsupial neonates are highly immature after a short gestation, although the anatomical and physiological precondition

is very similar in both groups, including the presence of an invasive trophoblastic tissue to initiate a highly effective placenta with the mother.

It has been hypothesized that the short gestation of marsupials should be interpreted as a reaction of the mother's immune system, which cannot cope with the fetal tissue that is genetically different from that of the mother (Lillegraven 1975, Amoroso and Perry 1975). However, it was later shown that maternal immune systems do not reject fetuses in macropodids (Walker and Tyndale-Biscoe 1978, Rodger *et al.* 1985). Instead, it seems that birth is induced by physiological processes of the fetus, and the short gestation and consequently longer lactational period are now considered to be a reproductive strategy to decrease the energetic investment of the mother in her young (Morton *et al.* 1982, Hsu *et al.* 1999).

The specialized trophoblastic tissue (Renfree 1993) of the last common ancestor of Placentalia likely possessed a similar kind of trophoblast as seen in living placental mammals, which may have allowed an enormous extension of intrauterine morphogenesis in eutherian mammals (Lillegraven *et al.* 1987). The elongated prenatal life (Hayssen *et al.* 1993, Vaughan *et al.* 2011) presumably resulted in the relative reduction of lactation time, faster reproduction, and ultimately increased population growth. Simultaneously with the gain of highly effective trophoblastic tissue, mammotrophic hormones may have enabled the mammary glands to nourish larger neonates. In a second phase of placental mammal evolution, higher metabolic rates may have evolved, which resulted in increased pre- and postnatal growth rates, further shortening reproduction time from conception to weaning and increasing population growth. An extended growth period facilitated more cerebralization among various clades of placental mammals, leading to larger relative brain sizes and more neurons and association pathways, which resulted in increased intelligence, enhanced longevity, and extensive play activities (e.g., Lillegraven *et al.* 1987, Barrickman *et al.* 2008, Isler and Van Schaik 2009).

An elongated gestation length in placental mammals, when compared with the mammalian ancestors, has the advantage of protecting the young for a long time in a fully separated and safe environment. The embryo/fetus can be nourished by the placenta for a long time and can reach, on average, a large prenatal body size. An obvious disadvantage is that in fluctuating environmental conditions, the pregnant mother is handicapped for a long time, and the intensive investment in uterine reproduction could result in the death of the mother and her unborn young (Tyndale Biscoe 2001).

Marsupials, at least in Australia, are exposed to a very fluctuating environment, and they can react more easily to changing conditions because of their reproductive strategy (i.e., short gestation and longer lactation). If the young die, a fast reproductive cycle enables the quick recovery of the population (Kirsch 1977). This is particularly obvious in kangaroos, which are able to "store" a dormant embryo in the uterus (diapause) and activate its development to replace a lost pouch young (Renfree 1993, Starck 1995). However, it has been pointed out that the ability to discard pouch young is irrelevant in many small marsupial species because they only raise one litter in their lifetime (Morton *et al.* 1982, Russell 1982).

The efficiency of reproduction is comparable among all mammalian groups, including monotremes. That is to say that per unit body mass, an equal or very similar number of offspring is produced (Hamilton *et al.* 2011). The greater *current* diversity of placental mammals over marsupials (Tab. 4.1) is not a representative time slot for the whole mammalian diversity produced throughout their evolutionary history. Evidence from the fossil record illustrates a great diversity over millions of years in both placentals and marsupials (Sánchez-Villagra 2013). Different life history strategies evolved to cope with different environmental conditions, which are not fully understood yet. Nevertheless, the evolution of different ontogenetic traits resulted in a fascinating variety of body forms found among monotreme, marsupial, and placental mammals.

4.16 Summarizing remarks

Mammalian biology is strongly characterized by features related to life history and reproduction. After cleavage, which is equatorial in therians but only occurs at the animal pole in monotremes, a blastocyst is formed. The trophoblast forms the outer layer of the blastocyst in all mammals (Fig. 4.4 F) and allows for the implantation of the embryo to the uterine wall in therians (Fig. 4.4 B–9). This establishes an intimate physiological connection between the embryo and the mother through the formation of a placenta and therefore supports therian viviparity.

The evolution of viviparity has had strong implications for early development in therians. The tertiary layer of the embryo, the non-mineralized eggshell, and the albumen that are present in monotremes are only present in part of marsupial intrauterine development and are completely absent in placental development with the exception of the rabbit. In addition, the amount of yolk in the egg cells of both marsupials and placentals is greatly

reduced compared with monotremes as nutrition is supplied directly in the womb.

In monotremes, the chorion fuses both with the vitelline sac and the allantois to form a choriovitelline and chorioallantoic membrane, respectively, which exchange gas and nutrition with the mother early in development. These membranes could be considered ancestral. The placenta of therian mammals is also formed by extraembryonic membranes (Fig. 4.5). Although a wide range of placenta types can be found in therians, a chorioallantoic placenta, which is especially effective in discharging nitrogenic waste from the embryo, is the most common among placentals (Fig. 4.6 E–J). Therians also show much diversity in the invasiveness of their placentas, ranging from the epitheliochorial placenta, in which almost no invasion of the uterine wall occurs, to endotheliochorial and hemochorial placenta types, in which the placenta is in direct contact with the maternal blood vessels (Fig. 4.6 A–D). Although the placental types overlap between marsupials and placentals, the placenta of placentals develops much earlier and generally shows more placental invasion.

Mammals also show a large diversity in their reproductive organs. Therians have a perineum, which separates the anus and the vagina (Fig. 4.2). Monotremes lack this structure and instead have a cloaca that is divided into a coprodaeum and a urodaeum. A scrotum is also only found in extant therians. Many marsupials have developed a pouch in which the young are kept in relative safety for a large part of their postnatal development. Monotremes and many placentals use a different approach to protecting their offspring by building nests.

Placentals show most diversity, both in life history strategy and morphology; gestation length ranges from short to very long, whereas it is always short in both monotremes and marsupials. Consequently, all monotremes and marsupials are altricial, but placental neonates range from highly altricial to precocial. As a result, placental developmental patterns have likely changed most from its ancestral origin. Reconstructing ancestral values by using squared-change parsimony analysis suggests that ancestral placental neonates were altricial, almost naked and blind, with evenly developed front and hind limbs (Tab. 4.4, Fig. 4.10).

Whereas most other primates are quite precocial, humans are secondarily altricial. This might be a result of the above-mentioned constraint in the size of the pelvic opening through which the head of the fetus must fit at birth (Fig. 4.13 P).

Marsupials are characterized by being the most altricial of all mammals. Because they are born at such an early stage of development, marsupials show specialized adaptations in many structures, including the limbs, the olfactory system, and the skull anatomy. In monotremes, the forelimbs are also at a much further stage of development, but their function is to cling to the fur of the mother, whereas marsupial neonates use the forelimbs to climb toward the mother's teat directly after birth. This adaptation is thought to pose some constraints on morphological diversity in marsupials. Marsupial forelimb morphology is generally conserved, and in contrast to placentals, no flippers or wings have developed in marsupials. Suckling at a very early stage of development also led to a conserved cranial ossification sequence and a highly derived tooth replacement pattern. However, although cerebral development is delayed in marsupials, this does not affect adult brain size, which is generally comparable with placentals.

Monotreme milk composition is generally similar to that of placentals, although it shares a high concentration of iron with marsupial milk, which is not the case in placentals. Marsupial lactation is highly specialized and the milk changes its composition through different phases of the development of the pouch young. If young of different ages suckle at the same time, both teats have a different milk composition. Furthermore, the nutritional value of marsupial milk is relatively low. Both are probably adaptations that reduce maternal investment. Weaning is a continuous process in all mammals and may take up to several months in certain placentals. Although the focus of maternal investment differs, with most investment occurring in utero in placentals and postnatally in marsupials and monotremes, the total amount of energetic investment is very similar across the three mammalian groups.

Overall, two approaches to reproduction can be distinguished in mammals. The first approach favors a large litter size with a high mortality rate and a relatively small parental investment per young. This altricial approach is exhibited most strongly by marsupials. The second approach, precociality, is present in certain placentals and favors a litter size which is quite small and a long childhood with intensive parental care, which therefore gives each individual young a much better chance of reaching sexual maturity. The striking differences between these two approaches have received much attention in the light of their respective diversity and evolutionary success. It has often been hypothesized that marsupial gestation is short due to the maternal immune system rejecting the fetus as it develops. However, this was tested and not confirmed for macropodids. Most likely, short gestation and a longer period of lactation represent a highly specific reproductive strategy that decreases maternal investment.

Therefore, it represents an extreme example of the diverse life histories adopted by mammals, facilitated by their unique developmental characteristics.

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